memoirs

3(1)

LIBRARY
DEC 7 1960
FISH & WILDLIFE SERVICE

3(1)

兵庫農科大学紀要
第三巻　第一号

MEMOIRS OF THE HYOGO UNIVERSITY OF AGRICULTURE

VOL. 3 NO. 1
MARCH 1960

(BIOLOGICAL SER. NO. 3)

A Comparative Study of the Chromosomes in Fishes with Particular Considerations on Taxonomy and Evolution

BY

SHYUNSAKU NOGUSA

PUBLISHED BY THE HYOGO UNIVERSITY OF AGRICULTURE
SASAYAMA. JAPAN

兵庫農科大学紀要
第三巻　第一号

MEMOIRS OF THE HYOGO UNIVERSITY OF AGRICULTURE

VOL. 3 NO. 1
MARCH 1960

(BIOLOGICAL SER. NO. 3)

A Comparative Study of the Chromosomes in Fishes with Particular Considerations on Taxonomy and Evolution

BY

SHYUNSAKU NOGUSA

PUBLISHED BY THE HYOGO UNIVERSITY OF AGRICULTURE
SASAYAMA, JAPAN

A Comparative Study of the Chromosomes in Fishes with Particular Considerations on Taxonomy and Evolution

By

Shyunsaku NOGUSA

(Biological Laboratory, Hyogo University of Agriculture)

(With 251 Figures in 6 Plates)

Contents

Introduction ..... (2)
Material and Methods ..... (4)
Results of Observations ..... (5)
Cephalochordata ..... (5)
A. Branchiostomidae ..... (5)
Cyclostomata ..... (7)
B. Eptatretidae ..... (7)
C. Petromyzonidae ..... (8)
Elasmobranchii ..... (9)
D. Triakidae ..... (10)
E. Dasyatidae ..... (10)
Teleostomi ..... (11)
F. Engraulidae ..... (11)
G. Salmonidae ..... (12)
H. Salangidae ..... (14)
I. Cyprinidae ..... (14)
J. Cobitidae ..... (22)
K. Siluridae ..... (23)
L. Bagridae ..... (24)
M. Echelidae ..... (24)
N. Muraenidae ..... (24)
O. Serranidae ..... (25)
P. Sillaginidae ..... (26)
Q. Parapercidae ..... (26)
R. Callionymidae ..... (27)
S. Gobiidae ..... (27)
T. Labridae ..... (32)
U. Aluteridae ..... (33)
V. Synanceiidae ..... (34)
W. Hexagrammidae ..... (36)
X. Cottidae ..... (36)
Y. Bothidae ..... (37)
Z. Pleuronectidae ..... (38)
Discussion ..... (38)
1. Cytological relation between the Prochordata and lower Vertebrates ..... (39)
2. Cytological features as seen in the orders of Pisces ..... (40)
3. Cytological features as seen in allied families ..... (42)
4. Cytological features between species within a family ..... (43)
5. Cytological evidence of the sex-determining mechanism in Pisces ..... (49)
6. Variation of the chromosome number due to a multiple chromosome formation ..... (51)
7. Occurrence of the univalent chromosomes ..... (52)
Summary ..... (53)
Literature Cited ..... (56)
Explanation of Plates 1~6 ..... (60)

Introduction

The behavior and morphological changes of chromosomes during phylogenesis has been a subject of great interest to cytologists, since the chromosomes take the most important role in the mechanism of heredity. Just fifty years ago, McCLUNG (1908) gave an address entitled "Cytology and Taxonomy" and stated in it his ideas of the close correlation that could be expected to exist between the chromosomes and the structural organization of organisms. Extensive progress of cytological knowledge has indicated that, so far as the higher animals are concerned, all evolutionary changes have arisen in the first place as changes in the chromosomes and that it is very valuable to use the cytological knowledge in establishing the relationship of taxonomical arrangements by comparing the complements, particularly the morphological details, of the chromosomes among related animals (WHITE 1954). There are striking contributions derived from the application of cytological methods to difficult taxonomic problems which are not always recognized by non-cytologists (WHITE 1957).

Class Pisces is a group of vertebrates in which chromosome surveys have progressed comparatively less than in other groups. The basic data for understanding the taxonomy and evolution have remained quite incomplete in every respect, as understood by referring to the chromosome monograph published by MAKINO (1956). It is quite desirable to make an accurate survey of the chromosomes in order to establish the mechanism of evolution in this field from both cytological and taxonomical standpoints. Mainly due to the fact that fishes are comparatively difficult material for cytological study, not much progress has been made previously in this direction; most of the marine fishes have been very inadequately investigated, and several are represented by blank spaces in cytological knowledge. Generally, the chromosomes of fishes are small, and in addition, they possess a high number of chromosomes: they are too small and numerous easily to permit a really detailed analysis. Further, there is some difficulty in collection of favorable material, particularly in securing the material in good season available for cytological study. Moreover, the preservation of chromosomes is very difficult in fishes in striking contrast to the case of most animals.

The Pisces is the most interesting group of vertebrates in relation to the evolution of animals, particularly when one is inquiring into the development of vertebrate animals. In this respect, exact knowledge on the chromosomes of the primitive groups of the chordate animals as Acrania and Cyclostomata is specially important for understanding the mechanism of heredity in evolution. At present, most of these animals have been very incompletely studied cytologically.

In connection with the phylogenetic importance, knowledge of the chromosomes in the lancelets belonging to the Cephalochordate seems to be of prime significance. The lancelets (*Amphioxus* or *Branchiostoma*) are of special zoological interest because of their phylogenetic relation to the Chordata. They have been described

as very primitive chordates having morphological characteristics closely related to vertebrates in many respects. In fact, except for the total lack of any part of the vertebrae and the poor development of the head structure. The lancelet is considered in a general way to resemble some ancient ancestor of the chordate animals. According to the phylogenetic conceptions advocated by certain theorists, the lancelets may be vertebrates of very primitive class, or a little specialized descendant of an ancestor shared by the vertebrates. Recently, GREGORY (1951) showed the relations between ascidians and *Amphioxus* in two alternative ways as follows:

Basic chordate——*Amphioxus*——Primitive fishes
↓ (Ostrachoderms)
Ascidians

Basic chordate——Primitive fishes——*Amphioxus*
(Ostrachoderms) ↓
Ascidians

Thus, in the first category a hypothetical chordate ancestor gave rise to *Amphioxus*, which in turn gave rise to primitive vertebrates in one direction and to the ascidians, by retrogressive evolution in another. In the second category both *Amphioxus* and the ascidians evolved from primitive vertebrates as two successive steps in retrograde evolution, with the main line evolving in another direction.

Next, the lowest primitive vertebrates are jawless animals known as Agnatha. Agnatha consists of two classes, Osteracordermi and Cyclostomata. The Osteracorderms are significant because they are oldest vertebrates so far known. The Cyclostomes have remained as the most primitive form not only in anatomical structure but also in embryological features. So far as the author is aware, no accounts have yet been published on the chromosomes of these animals. Thus a thorough investigation of the chromosomes is also a subject which received attention in relation to the evolutionary mechanisms of animals.

The cytological sex-determining mechanism of Pisces has been a matter of discussion during these thirty years among both cytologists and genetists from both genetical and evolutional view-points. Though considerable work has been done genetically on the sex-determining mechanism in fishes, the question of the sex-chromosomes has not yet been decisively cleared up in any species so far. It has been emphasized by MAKINO (1934), Wickbom (1943) and WHITE (1954) that the sex-chromosomes of Pisces as well as Amphibia are the state of lowest differentiation in evolution. According to their views, the sex-chromosomes of the lower vertebrates show no morphological differentiation from the autosomes. In striking contrast to the above view, the present author furnished for the first time definite cytological evidence regarding the sex-determining mechanism by establishing a clear-cut XY mechanism in a species of the Gobiidae, *Mogrunda obscura* (NOGUSA 1955), and in a species of Cottidae, *Cottus pollux*, and contributed firm evidence to the cytological demonstration of male heterogamety in teleost fishes.

Thus, it is evident that the basic data for understanding the evolution of the

mechanism of heredity in fishes are very incomplete. The present author has been engaging for about fifteen years in a chromosome survey of Pisces with a hope to contribute something in this cytologically unexplored field with special concern to the phylogenetic significance of cytological characters. In the following descriptions, the author wishes to deal with the chromosomes of 64 species of fish which belong to one family of Cephalochordata, two families of Cyclostomata and 21 families of Teleostomi. There is special reference to any cytologically detectable difference between the chromosome sets of related species for understanding of the evolutional mechanism of fishes, and further special reference to the sex-determining mechanism of fishes in relation to taxonomy and evolution.

Acknowledgement: Before going further, the author wishes to acknowledge here his great indebtedness to Dr. Sajiro Makino, Professor of Zoology at Hokkaido University, who suggested the problem of this study and who has lent almost continuous encouragement. The author is very greatful for his expert supervision, for many valuable suggestions and for his careful direction during the course of this work. Futher the author's hearty thanks should be extended to Dr. T. Mori, for collection and identification of the fishes herein dealt with, and for his kind help in various ways. Also, the author is much obliged to Professor Kiyoshi Suzuki and Dr. Osamu Minouchi for their kind cooperation in observation and valuable advices with discussion. In especial he wishes to thank Dr. S. Miyake, President of this University, who has furnished him the opportunity of publishing the work.

Material and Methods

The species on which the chromosome survey was based are 64 in total: they are represented by 24 families which are included in four groups of lower vertebrates viz., Cephalochordata, Cyclostomata, Elasmobranchii and Teleostes. The names of species and their taxonomical relationships are as shown in Table 10. The classification system here considered is based on the first edition of "Fish Morphology and Hierarchy" edited by Matsubara (1955).

The specimens used for study were collected in various districts in Japan. Collecting localities cover various districts of Hokkaido (Abashiri, Chitose, Ishikari, Lake Shikotsu), Akita Prefecture (Lake Towada), Niigata Prefecture (Niigata City), Fukui Prefecture (Oono City), Shiga Prefecture (Lake Biwa, Yōkaichi City), Wakayama Prefecture (Owashi City and Tanabe City), Osaka (the vicinity of Osaka City), Hyogo Prefecture (Amagasaki City, Akashi City, Awaji Island, Sasayama, Ichijima and so on), Tottori Prefecture (Lake Koyama), Shimane Prefecture (Lake Shinji), Hiroshima Prefecture (Saizaki-cho), and Kyushu (Oomuta City, Yanagawa City and Ariake Bay).

Gonads derived exclusively from adult males furnished the material for investigation. The testes were taken out by vivisection immediately after capture and put into fixatives as soon as possible. The fixatives used were Champy's solution re-

commended for the study of the Piscian chromosomes by MAKINO (1934) and its modifications devised by the present author: they are (1) Champy's mixture containing 3.5% potassium bichromate, (2) the same diluted with distilled water, and (3) the same diluted one-third by adding distilled water. All fixatives gave favourable results, particularly the solution of the second formula proved excellent for the preservation of several fresh water teleosts. Subjected to the usual paraffin method, the sections were cut mostly 4 to 5 micra thick, or occassionally 10 micra, and stained with HEIDENHAIN's iron-haematoxylin with a counter stain of light-green, partly according to Feulgen's method. All of the figures were drawn with the aid of a camera lucida at magnifications of 3200, 3500, 3800 and 4500 times.

The species dealt with in this investigation are arranged in the current taxonomical system: they are represented by 64 species covering 24 families in 12 orders, as listed in Table 10.

Results of Observations

Subphylum Cephalochordata

This subphylum comprises about 30 species of small fishlike marine animals (*Branchiostoma, etc.*), commonly called the lancelet or *Amphioxus* that inhabit tropical and subtropical seacoasts. In connection with its phylogenetic importance, knowledge on the chromosomes of lancelets seems to be of special significance for any theoretical consideration regarding their evolution.

The cytological study of lancelets has been carried out by classical investigators, such as VAN der STRICHT (1895, 1896), SOBOTTA (1897) and CERFONTAINE (1905a, b). These authors dealt with the course of ovogenesis or of egg-development in *Amphioxus lanceolatus*. It is needed therefore to investigate the chromosmes of lancelets with the application of modern technique in order to provide accurate cytological knowledge in relation to their evolutional development. In response to this need, the present study has aimed at obtaining an exact account on the chromosomes of the Japanese lancelet.

A. Family Branchiostomidae

1. *Branchiostoma belcheri* GRAY

The germ cells of this species are extremely small in size. A careful chromosome count showed that the diploid number of this species was constantly 32 in the male (Figs. 1–3). The majority of the diploid members seem to be of typical rod-type, judging from their short rod-shape and the taper end. The diploid complement contains a pair of elements dissimilar in length, the one being remarkably larger than the other which is extremely small in length. The elements other than these two are nearly identical in size. Judging from their remarkable size-difference the two chromosomes seem to represent an XY-pair of the sex-choromosomes, according to the general rule on the sex-chromosome mechanism. Then the X- and Y-elements are very remarkable in appearance in the male diploid complement on account of

their size-difference.

The choromosomes found in the first meiotic metaphase are very clearly observable because of the reduced number. The primary spermatocyte contains at metaphase 16 well-defined bivalents; they consist of 15 autosomal bivalents and a striking heteromorphic bivalent composed of an X and a Y, as shown in Figures 4 to 5. The XY bivalent always lies in the most peripheral region in the equatorial arrangement. The X conjugates with the Y in the form of an end-to-end association: the rod-shaped X connects in its extremity with the Y of small size in a linear series, as seen in the side view of the spindle (Fig. 6). The XY-bivalent stands nearly perpendicular to the equatorial plate at metaphase. The X segregates from the Y in the first division, resulting in the production of two kinds of secondary spermatocytes in respect to the distribution of the X- and Y-elements. Every secondary spermatocyte at metaphase always shows 16 chromosomes. The secondary spermatocytes show a distinction between two sorts, one containing a large X and the other a small Y (Figs. 7–8).

On the basis of the above observations, the conclusion was reached that the number of choromosomes in the Japanese lancelet in 32 in $2n$ (♂) and 16 in n, and that the chromosome complement consists of 15 pairs of rod-shaped autosomes and an XY pair composed of a large X and a small Y. The X and Y are rather clearly distinguishable from the autosomes because of their characteristic size-difference.

The investigation of chromosomes in the Prochordata including the related species has been studied by earlier investigators, such as Boveri (1890), Hill (1890), Julin (1893), Bancroft (1899) and Minouchi (1836) in the Tunicata, Van der Stricht (1895, 1896), Soeotta (1897) and Cerfontaine (1905a, b) in the Acrania (cf. the list compiled by Makino, 1956). The majority of these pioneer investigators directed their attention towards the maturating phenomena of the egg cell or the the course of egg development. It is, therefore, evident that their results are quite unsatisfactory by present-day standards. Of noticeable importance is the paper published by Minouchi (1931) who studied the chromosomes of *Tethyum plicatum* (Tunicata); he reported the haploid number of 16 in this species, though he found no sexual difference of chromosomes. Recently, Zwillenberg and Zwillenberg (1954) studied the chromosomes of three species of tunicates; they reported that the haploid numbers were 14 in *Aplidium,* 8 in *Pyura* and 9 in *Sidnyum,* and that there occurred no sexual difference of chromosomes in any form.

The present study seems to be the first to record the sex-chromosome mechanism in a species of the Prochordata. As given in the foregoing pages, the Japanese lancet, *Branchiostoma belcheri,* showed the diploid number of 32 in the spermatogonium (♂ 2n) and the haploid number of 16 in both primary and secondary spermatocytes, with the sex-choromosome mechanism of an XY-type. Obviously, the chromosome number of this species is higher in comparison with that of a related form, *Amphioxus lanceolatus,* reported by earlier investigators (Van der Stricht 1895, 1896, Sobo-

TTA 1897, CERFONTAINE 1905a, b).

Subphylum Vertebrata

Class Cyclostomata

According to the general conception of phylogeny, the cyclostomes are the most primitive in surviving vertebrates in many anatomical structures. The class Cyclostomata consists of two orders, Mixinida (Hag-fishes) and Petromyzonida (Lampreys). They are considered to be modified and degraded descendants of the oldest known forerunners of vertebrates. Moreover it is suspected by some authors that the ammocoete larva of the lamprey approaches rather close to the condition of the ancestral chordata. Some have gone so far to suggest that the amphioxus, as we find it to-day, may be a permanent larva of some vertebrates related rather closely to the cyclostomes. This view is supported to some extent by the fact that the amphioxus is so obviously degenerative in other respects.

In connection with the above facts, the chromosome survey of this group is also interesting and important in relation to the evolutional change of vertebrates. So far as the author is aware, there are four classical papers in which the chromosomes of two cyclostomes, *Bdellostoma burgeri* and *Myxine glutinosa,* have been described by RETZUS (1890) and SCHREINER (1904 a, 1904 b and 1908). These earlier investigations, however, could not furnish any accurate accounts of the chromosomes. Recently the present author has had a chance to investigate the chromosomes of two species of hagfishes, *Entatretus burgeri* and *E. okinoseanus.* In addition, it is very fortunate to present here the data obtained from the sand eight-eyes lamprey, *Entosphenus reissneri.*

B. Family Eptatretidae

2. *Entatretus burgeri* (GIRARD)

Along the Pacific coast of Japan, the hagfishes (*Entatretus burgeri*) are abundant distributing from Boshu, Chiba Prefecture to the sea of Japan where they occur rather commonly in the coast off southern Korea. The testicular materials for the present observations were secured by the dragnet fishery, in Owashi, Wakayama Prefecture.

Careful observations revealed that the male diploid complex contained 48 chromosomes in the spermatogonial metaphase (Figs. 9—10). The chromosome complex is typically of isomorphic nature, every element being uniformly short rod-shaped with a slight variation in size.

The majority of the chromosomes at the first meiosis are of ordinary bivalent in structure (Figs. 11—12). Of bivalents found in a peripheral zone of the equatorial plate, there are a few ones, 2 to 6 in number, which are sharply distinguishable from others by having a remarkably distinguishing feature in their morphology: they exhibit a distinct dumb-bell shape, showing a conspicuous transverse suture in each middle region. They seem to be nearly identical in size and come together to closely associate with each other in a feature like the so-called secondary association. The association of five such bivalents was most prevalent (52%), four bivalents the

next. The frequent occurrence of the secondary association is shown in Table 1.

Table 1. Association type of the dumb-bell shaped bivalents

No. of bivalents under associaton	6	5	4	3	2
Frequency in %	1.5	52	30	11.3	4.3

The secondary spermatocytes show 24 chromosomes at metaphase (Fig. 13). The secondary association is also remarkable in this stage displaying a peculiar feature.

3. *Entatretus okinoseanus* (Dean)

The present species commonly occurs in the deep waters along the Pacific coast of middle Honshu. Many male individuals were obtained from the same place as the former species in March and April, 1956.

The diploid number of chromosomes as counted in the spermatogonial division was determined to be 46, two less than the number found in the previous species, *E. burgeri* (Figs. 14—15). The general chromosomal features of this species are apparently alike to those of the preceding one: all chromosomes uniformly show a terminal centromere structure. It seems impossible to identify the homologous pairs because of the prevalence of chromosomes with slight differences.

The haploid number, 23, was clearly determined in both the primary and secondary spermatocytes (Figs. 16—18). As observed in the previous species, there are 2—5 bivalents which occur in close association just like the so-called secondary association. They are dumb-bell in shape. Considered from their shape and structure, it is evident that these bivalents are of similar nature to those observed in the former species. The frequency of 3—4 association types was found to be 71.2% (Table 2).

Table 2. Association of dumb-bull shaped bivalents

No. of bivalents showing association	5	4	3	2
Frequency in %	21	40.7	30.5	7.8

C. Family Petromyzonidae

4. *Entosphenus reissneri* (Dibowski)

The present species is a small lamprey which inhabit rivers and lakes connected both with Pacific Ocean and the Sea of Japan. A number of fishes were obtained in Oono City, Fukui Prefecture, in March 1953.

Every specimen under study was not available for the study of the maturation division, due to the unfavorable season of collection. It is surprising to see that the present species shows a number of chromosomes very higher than those of the above two species. The extreme smallness in cell-size associated with the high number of chromosomes make the study very difficult, particularly in determining the accurate chromosome number. The spermatogonial chromosomes were found to be 94 to 96

in number (Figs. 19—20). The members constituting the diploid complement seem to be of telomitic nature, every element appearing as a uniform and short rod-shaped structure with a slight variation in size. The chromosome complex of this species is therefore to be of the isomorphic type.

Referring to the literature, there are found four papers reporting the chromosomes of the Cyclostomata published by pioneer authors: they are by RETZIUS (1890), and SCHREINER (1904 a, 1904 b, 1908). They dealt with the chromosomes *Bdellostoma burgeri* and *Myxine glutinosa* without giving any accurate accounts. The chromosomes of two hagfishes herein considered, *Eptatretus burgeri* and *E. okinoseanus,* are nearly alike in their complement, not only in their number but also in other respects of morphology; the number of chromosomes in *E. burgeri* is 48 in diploid and 24 in haploid, while *E. okinoseanus* shows the diploid number of 46 and the haploid number of 23. The chromosome complex is typically isomorphic and telomitic in nature in both species. The chromosomes of *Entosphenus reissneri* are also characterized by possessing the karyotype composed of rod-shaped elements, notwithstanding the number of chromosomes is considerably different from the above species, being 94 to 96. The conclusion is made that throughout the meiotic stages of spermatogenesis, there is no evidence for the presence of particular chromosomes in the three species here under study.

The secondary association which occurs as a result of the paring of two or more bivalent chromosomes in meiosis has been widely known in many plant forms. In animals, clear-cut evidence for the seconary association has been reported by MAKINO (1948) in a species of the Neuroptera, *Palpares pardus* var. *asanai.* This seems to be the first and sole report to record in animals. As described above, the first and second meiotic metaphases of the two cyclostome species, *E. burgeri* and *E. okinoseanus,* show without exception a few bivalents, 2 to 6 in number, which came together to closely associate with a feature like the so-called secondary association. With ragard to the cause of the secondary association, nothing is to be stated here; further statements may be made after the accumulation of data of similar nature in related forms.

Class Chondrichthyes

Subclass Elasmobranchii

The Elasmobranch fishes are vertebrates of the lowest class having complete and separate vertebrae, movable jaws and paired appendages. The group is of special interest to anatomists, because it occupies a position between the Cyclostomes and Teleostes in anatomical points of view.

Since the pioneer study by KASTSCHENKO as early as 1890, the cytological studies of the Elasmobranchii has been undertaken by a number of earlier authors from the end of the last to the beginning of the present century (Makino's list 1956): they were directed their interest towards elucidation of maturating phenomena in the sperm and egg cells. They made only an approximate calculation of the chromo-

some number in these animals. With the application of modern technique, the chromosomes of two species of Elasmobranchs, the shark and skate, were studied by Makino (1937) who furnished valuable data for understanding the evolutionary relation of fiishes. Recently, Matthey (1947) has reported on the chromosomes of a species of the Squalidae. Here, the present author has had a chance to investigate the chromosomes of two species of Elasmobranchus, *Mustelus manazo* and *Dasyats akajei*.

D. Family Triakidae

5. *Mustelus manazo* Bleeker

This is a species of the commonest sharks in Japan, occurring in shallow bay throughout the entire coast of Japan. The material on which the following observations were based, consisted of testes from five specimens obtained in Kariya, Awaji Island, in September and October of 1956.

The spermatogonial chromosomes were examined in several clear metaphase plates. After the carefully counting it was decided without any confliction that the number of chromosomes in a spermatogonium is 72 (Figs. 21—22). The chromosome complement of this form consists of two groups; atelomitic chromosomes with median or submedian centromeres which occupy a peripheral zone of the equatorial plate are nearly 12, or more, in number. The remaining 60 elements seem to be of telomitic nature.

The chromosome count at the first metaphase shows 36 chromosomes with bivalent structure having various shapes and sizes (Figs. 23—24).

In conclusion, the present species possesses 72 chromosomes in diploid and 36 in haploid, and the formula of the male chromosome complex is given as 12V's+60r's.

Generally speaking, the chromosomes constitution of Elasmobranch fishes is not simple but a complicated one, with a rather high number of chromosomes. Working with *Squalus suckleyi* (Squalidae), Makino (1937) reported 62 diploid and 31 haploid numbers in that species. The diploid complement consists of several types of about 20 atelomitic elements and the remaining rod-shaped ones. In comparison with *Mustelus manazo* here dealt with there is a general similarity between the two species in the morphology of chromsomes. Matthey (1945) reported in *Scyliorhinus catula* belonging to the Squalidae the chromosome number of 31 in haploid.

E. Family Dasyatidae

6. *Dasyatis akajei* (Müller et Henle)

This is a sting ray, very common on the coasts of southern Japan. It is viviparous, the breeding season occurring in summer. The testes from three individuals comprise the material for the present study.

The number of chromosomes was 84 (*2n*) in the spermatogonial complex (Figs. 25—26). Approximately 20 elements in a rough calculation are of metacentric or submetacentric structure and occupy the peripheral zone of the equatorial plate, surrounding the remaining telocentric ones which are scattered in the central region, though it was difficult to determine with certainty the shape of individual chromosomes.

The haploid chromosomes observed in the primary spermatocytes were 42 (n) in number (Fig. 27–28). At anaphase of the first division, all the chromosomes divide synchronously each into equal halves, and there is no particular element which is outstanding in their shape or behavior. It is thus apparent that the present species possesses 84 chromosomes in diploid and 42 in haploid, showing the male chromosome formula as follows: 20 (V's or J's)+64r's.

On looking up the literature, two classical paper by MOORE (1985) and FARMER and MOORE (1904) on the chromsomes of two species of the Rajidae, *Raja macrorhynchus* and R*aja maculata* is referable, According to these authors, the diploid number of chromosomes in the male is 24 and the haploid number is 12 in both species. KASTSCHENKO (1890) reported about 50 chromosomes in the first division of the egg of T*orpedo ocellata,* a species of electoric rays belonging to the Torpediae, but to the same order Rajida. The chromosome number in the above three species is astonishingly low as compared with the results reported by recent authors. According to MAKINO (1937), the number of chromosomes in *Raja meerdervoortii* (Rajidae) is 104 in diploid and 52 in haploid. The diploid complex is composed of telomitic rod-shaped chromosmes. Comparison showed that the chromosomes of *Raja meerdervoortii* are surprisingly dissimilar from those of *Dasyatis akajei,* not only in number, but also in other morphological respects, despite of that they belong to the same order Dasyatida in their taxonomical relation. The karyotype of Dayatidae is rather closely related to that of the Order Laminida than to that of Rajidae,

Class Osteichthys

Subclass Teleostomi

Order Clupeida

The cytological studies of this order has so far been made on the following two families, Esocidae and Salmonidae; there are cytological records in two species for the former and in 19 species and 4 hybrids for the latter. The present study records the results of studies on one species of the Engraulidae, six species of the Salmonidae and one species of the Salangidae.

F. Family Engraulidae

This family has never been cytologically dealt with previously. The present paper describes the chromosomes of one species, *Engraulis japonicus* of the Eengraulidae.

7. *Engraulis japonicus* TEMMINCK et SCHLEGEL

The present species is very common along the shores of Japan. The material was obtained in Owashi, Wakayama Prefecture, in February, 1956.

The number of the chromosomes was decided as 48 ($2n$) in the spermatogonial metaphase plate: all are of rod-type characterized by terminal centromere in each (Figs. 29–30).

The haploid chromosome number of 24 was found in the primary spermatocytes (Fig. 31).

To summarize, the present species is characterized by the chromosome formula of 48r's.

G. Family Salmonidae

From the systematic viewpoint, it can be said that the Salmonidae is a group of fishes which provide many taxonomical difficulties that disturb ichtyologists with regard to the distinction of the species.

The cytological investigations of the Salmonidae have been progressively carried out in many species. Reference to the new list by Makino (1956) shows that the chromosomes of 19 species and 4 hybrids have been reported in this family. Earlier accounts published by Bohm (1891), Blanc (1894), Behrens (1898), Oppermann (1913) and Mrsic (1923) gave no evidence satisfactory at present-day standards. Lately, a series of cytological investigations has been published in this family by Prokofieva (1934), Makino (1937), Pomini (1939), Svärdson (1941, 1945), Kupka (1948), Bungenberg (1955) and Boothroyd (1957) with satisfactory results.

Most of these studies dealt with the chromosomes during egg-cleavage. The chromosomes of the cleavage mitosis appear in an elongated thread-like form with unusual indefinite outline, representing the frequent occurrence of fragmentation. As suggested by Wilson (1925), the final confirmatory evidence would be obtained through a study of chromosomes in germ cells. Svärdson (1941, 1945) studied the morphology of the chromosomes of several salmonids, and concluded that the Salmonidae are regarded as being in polypolid relationship. The present paper records the results of observations of six species of the Salmonidae in the course of spermatogenesis.

8. *Oncorhychus nerka* (Walbaum)

The native locality of land-locked forms of this trout are Lake Akan in Hokkaido. About 1894 the fishes were introduced to other lakes and ponds in various localities; they have sucessfully propagated at the present time. The material employed in this study consists of the testes obtained from five adult specimens collected in Lake Shikotsu, Hokkaido.

The material proved to be insuffiicient for a complete study of the spermatogenesis. Every first spermatocyte studied showed at metaphase 54 chromosomes (Fig. 32). The chromosomes are all bivalent in structure.

9. *Oncorhynchus rhodurus* Jordan et McGregor

This species occurs in upper reaches of the river of middle and south Japan and Lake Biwa. The specimens for this study were collected from the streams adjoining the vicinity of Sasayama, Hyogo Prefecture.

The haploid number of chromosomes was determined as 50 in the primary spermatocytes (Fig. 33).

10. *Oncorhynchus masou* (Brevoort)

This is a common species in northern Honshu and Hokkaido. The material from andromonus fishes consisted of a pair of testes obtained from a male specimen in

near Abashiri, Hokkaido. The land-locked forms were collected in Unazuki, Toyama Prefecture.

The haploid number of chromosomes was observed to be 50 in the primary spermatocyte in both of the andromonus fishes and land-locked fishes (Figs. 34–35). The chromosomes are all ordinary bivalents in structure, each composing two equal components; there is no bivalent consisting of unequal components.

11. *Oncorhynchus keta* (WALBAUM)

The present species is especially abundant in Hokkaido, and ranges to Kamchatka, Alaska and northern California, being very familiar to people by the name of salmon. Testicular tissue derived from a specimen obtained in Ishikari, near Sapporo provided the material for study.

The haploid number of chromosomes was 50 based on the observations of the primary spermatocytes (Fig. 36). The haploid complex consists of 50 bivalents, each being made up of two equivalent components giving an ordinary structure.

Previously, MAKINO (1937) reported 78 chromosomes in the diploid group of this species. According to him, the diploid complement consists of telomitic and atelomitic chromosomes. The chromosome number established here is in disagreement with that given by MAKINO who studied the number in the primordial germ cells of embryonal gonads.

12. *Salmo irideus* GIBBONS

This trout was introduced from California to Japan and has continuously been bred with successful results. Testicular material obtained from three specimens was secured in Yase, near Kyoto.

There are 104 chromosomes in every spermatogonial metaphase studied (Fig. 37). The diploid complex is composed of various kinds of atelomitic chromosomes, at least 14 or more in number, and the remaining telomitic ones.

The haploid number, 52, was determined with rather accuracy (Fig. 38). In the polar views the bivalents assume a rounded or somewhat elongated outline.

As early as 1898 BEHRENS studied the chromsomes of *Salmo irideus*, reporting that the number of chromosomes was 2*n*, 24. MRSIC (1923) gave the chromosome number of this species as 2*n*, 12. Recently the chromosomes of *Salmo irideus* have been studied by BUNGENBERG (1955), who observed 60 chromosomes during the course of cleavage of which at least 40 chromosomes have median or submedian centromeres. BOOTHROYD (1957) determined the diploid chromosome number as 56 in this species. The present study was carried out with the germ cells, and yielded the results showing that the diploid number was 104 in the spermatogonia and the haploid number was 52 in the primary spermatcytes. The chromosome counts given by the former authors are astonishingly low as compared with the results by the present author. The evidence seems to be interpreted in two possible ways as follows: firstly, the former investigators were based on the squash method, while in the present investigation the usual sectioning method in combination with HEIDENHAIN's iron-haemato-

xylin stain was adopted. Secondarily, Bungenberg and Boothroyd used the embryonal tissue for study: it is rather difficault to examine the chromosome number in embryonal tissues of fishes.

13. *Salvelinus fontinalis* (Michii)

The brook trouts, now raised in northern Japan, were introduced from eastern parts of the United States. The specimens for the present material were furnished through the courtesy of the Unazuki Fish Farm in Toyama Prefecture.

The haploid chromosomes observed in the primary spermatocytes were 50 in number (Fig. 39),

H. Family Salangidae

No species belonging to the Salangidae have been cytologically studied prior to the present study.

14. *Salangichthys microdon* (Bleeker)

The present species widely distributes ranging from northern Honshu to Kyushu, and in Korea. The specimens collected in Lake Shinji, Shimane Prefecture provided the material for study.

The haploid number of chromosomes was determined as 28 in every primary spermatocyte studied (Figs. 40—41).

Order Cyprinida

Referring to the literature (Makino's list 1956), the chromosomes have hitherto been reported upon in two families, Cyrinidae and Cobitidae. In the present study an additional contribution may be mad by a chromosome survery of the following four families, Cyprinidae, Cobitidae, Siluridae and Bagridae.

I. Family Cyprinidae

In the family Cyprinidae, Makino (1934, 1935) was the first to give accurate accounts on the chromosomes of two species, *Cyprinus carpio* and *Carassius auratus* (*carassius*). Additionally Barigozzi (1937) made also a study of the chromosomes of *Cyprinus carpio specularis*. Then, the chromosomes of six cyprinoid fishes and one hybrid have been reported by Makino (1939, 1941), those of one hybrid by Makino, Ojima and Matsui (1955), those of ten species by the present author (Nogusa 1943, 1951, 1955), and those of one species by Matsui, Ojima and Nogusa (1951).

15. *Rhodeus ocellatus* (Kner)

This species is a small fresh-water fish inhabiting Lake Biwa of Japan, as well as the land waters of Korea and China. Five pairs of testes obtained in Amagasaki near Osaka provided the material for this study.

Careful study of several equatorial plates excellently preserved resulted in that the diploid number of chromosomes in the spermatogonial metaphase was invariably 44 (Fig. 42—43). In the diploid complement, a pair of V-shape showing submedian centromere and of large rod-shaped members are conspicuous by their outstanding feature. The remaining 40 chromosomes seem to be of telomitic type, having each a simple rod-shape with tapering end. They show a gradual reduction of length

from the rod to the spheroidal one.

The haploid number of chromosomes was determined as 22 in the primary spermatocytes (Fig. 44). One bivalent assuming the form of a V and the largest one is clearly distinguishable from other bivalents. There are again observable 22 chromosomes in the secondary spermatocytes (Fig. 45).

16. *Acheilognathus rhombea* (Temminck et Schlegel)

Acheilognathus rhombea on which this study was based, is a common fresh water teleost. It has a wide distribution through the southern part of Japan in Honshu and Kyushu. Testicular material derived from 20 specimens provided the material for the present study.

Specially noticeable is the occurrence of *three different karyotypes* in the present form: they are designated as the 4V-, 2V- and NO V-type according to the number of V-shaped multiple chromosomes. The chromosome types will be described in detail in the following.

(1) *4V-type complex :* This karyotype is remarkable by showing four V-shaped chromosomes in the diploid complex. The diploid number of chromosomes observed in the spermatogonial metaphase was clearly 44 (Fig. 46—47). Forty elements in the complement are rod-shaped, tepering towards their ends ; they show a slight variation in length. Four chromosomes in the complement assume a V-shape characterized by a median or submedian centromere. This finding is substantiated by the fact that two of twenty-two bivalents found in the primary spermatocytes are provided with metacentric centromeres (Fig. 48). In all probability they originate from the four V-shaped elements in the diploid group. Similarly, the second metaphase complex also shows two chromosomes of a metacentric nature (Fig. 49).

(2) *2V-type complex:* The spermatogonial complex show 46 chromosomes the number being two more than that of the former type (Fig. 50—51). The diploid complement of this type is remarkable in that it includes two V-shaped elements each characterized by a metacentric centromere, in addition to 44 chromosomes of a simple rod-type, which differ very slightly in length. Comparison shows no visible differences in shape and size of each element between the former type and the present one. If it be assumed that each of the V-shaped chromosomes may be double resulting from centric fusion, the apparent variation in the chromosome number occurring in this type may be explicable as having resulted on the basis of a multiple formation.

Both the primary and secondary spermatocytes distinctly show 23 elements at metaphase, one of them V-shaped possessing a metacentric centromere (Figs. 52—53).

(3) *NO V-type complex:* There is no evidence for the occurrence of the V-shaped element in the diploid complement of this type, which shows a diploid number of 48 (Figs. 54—55). In striking contrast to the above two types, all members in the diploid complement of the present type are distinctly rod-shaped. The haploid number, 24, was clearly observed in both the primary and secondary spermatocytes (Figs. 56—57).

Close examinations revealed in the present specimens neither abnormal divisions nor cells undergoing pycnotic degeneration during spermatogenesis. Further, no particular chromosome such as the sex-chromosome was detected in any of the specimens so far studied.

The chromosomal relationship of the three types: The present invetigation made it clear that there are three different populations having different karyotypes in *Acheiognathus rhombea*. They are described as the 4V-type, 2V-type and NO V-type, according to the number of the V-shaped elements. The chromosome formula is given for each type as follows:

4V-type: 4V's+40r's=2*n*, 44
2V-type: 2V's+44r's=2*n*, 46
NO V-type: 48r's=2*n*, 48

As mentioned in the foregoing descriptions, the numerical and morphological differences in the chromosomes between the 4V-type and the 2V-type may be accounted for on the basis of the formation of multiple chromosomes through centric union. If two V-shaped chromosomes of the 4V-type be conceived of as four rods, the total number becomes 46, which is the number characteristic of the 2V-type. A similar relationship is also found to occur between the 2V-type and NO V-type. The chromosomal relationship occurring in the three types as above may be readily understood by reference to Figures 58, 59 and 60, in which the diploid chromosomes are placed in paired alignment of supposed homologous mates in the approximate order of size.

As described above, the karyotype of *Acheilognathua rhombea* are classified into three distinct types according to the number of V-shaped chromosomes. Closer observations revealed that various karyotypes occurred in a single individual. In other words, each individual is not characterized by a single karyotype. The results of a statistical observations on the frequency in occurrence of karyotypes per individual are summarized in Table 3.

Table 3. Occurrence of three karyotypes per individual

Karyotype obv.	4V	2V	4V & 2V	4V & NO V	4V & 2V & NO V	Tatal
No. of specimens obv.	2	2	4	1	1	12

The numerical data seem to show that there is tendency for individuals characterized by two or three karyotypes to be more frequent than those having single karyotype, and further that there is no individual characterized by a NO V-type only, so far as the present observations are concerned.

The occurrence of the three types of the haploid number was next observed as shown in Table 4.

Referring to Table 4, it is apparent that the majority of specimens observed show two or three types with respect to the haploid number in the primary

Table 4. Occurrence of three haploid numbers

Haploid number obv.	22	22 & 23	23 & 24	22 & 23 & 24	Total
No. of specimens obv.	1	9	3	7	12

spermatocytes. Only a single individual is characterized by a single type of the haploid number of 22.

Next the distribution of the haploid numbers in three types was examined in testicular cysts. Twenty-five testicular cysts showing a number of dividing figures of primary spermatocytes were selected for study. The results are as shown in Table 5.

Table 5. Distribution of the haploid numbers in 25 testicular cysts

Haploid number	22 & 23				22 & 23 & 24				23 & 24		
Ratio in occur.	1 : 1	2 : 1	3 : 1	Total	1:2:1	1:1:2	2:1:1	Total	1 : 1	2 : 1	Total
No. of cysts	2	4	4	10	2	3	4	9	3	3	6

From the data of Table 5, it is evident that every testicular cyst contains cells showing two or three haploid numbers, and further that the different types of the haploid number occur in inconstant ratios.

17. *Acheilognathus limbata* (Temminck et Schlegel)

This species widely distributes in the river and streams of the western part of Japan. The testes from male specimens obtained in Yōkaichi, Shiga Perfecture provided the present material.

The general morphological feature of chromosomes is quite similar to that observed in former species, *Acheilognathus rhombea,* the total number of the spermatogonial chromosomes being 44 (Figs. 61—62). The diploid complement contains at least 4 V-shaped chromosomes, each being characterized by a submedian centromere with dissimilar arms, while the remaining ones are all telomitic rods showing gradual diminution of size.

The metaphase plate of the primary spermatocyte shows well-defined 22 bivalents with indisputable clearness (Fig. 63). The V-shaped bivalents are very distinctive amongst the others.

18. *Acheilognathus cyanostigma* Jordan et Fowler

This is a common fresh water teleost along the shore of Lake Biwa and in the vicinity of Ogaki, Gifu Prefecture. The present material was derived from the testes of male specimens obtained in the nighbourhood of Osaka.

The karyotype of this species assumes a close resemblance to that of the above two species. That is, the spermatogonial complex shows 44 chromosomes of which two pairs are atelomitic V-shaped, while the remaining ones are telomitic rod-shaped (Figs. 64—65). The haploid chromosome number, 22, was observed with clearness

in the primary spermatocyte (Fig. 66).

19. *Pungtungia herzi* Herzenstein (*Pungtungia hilgendorfi* Ishikawa)

This is a fresh-water fish found southward from the middle Japan and Korea, and spawns in May. Several males collected in Teramae, Hyogo Prefecture, furnished the material.

Only the spermatogonia were available for study in the present material. Counting showed that the spermatogonial chromosome contained invariably 50 in diploid (Figs. 67—68). The diploid complement shows at least 4 V-shaped chromosomes, each being characterized by a submedian centromere with dissimilar arms. The members of the remaining 46 chromosomes are regarded as telocentric in their structure, since they assume a simple rod-shape with a taper end.

20. *Sarcocheilichthys variegatus* (Temminck et Schlegel)

This fish occurs as a native in the rivers and lakes of southern Japan, but have now been introduced into various parts of this country. The specimens used as material were collected in Lake Biwa and in the vicinity of Sasayama, Hyogo Prefecture.

Every spermatogonial metaphase shows 50 distinct chromosomes arranging radially in the equatorial plate (Fig. 69), all being telocentric rods in shape. Four among them (two pairs) are in striking contrast to the others on account of their very large size. The haploid chromosome number was found as 25 through observations of both primary and secondary spermatocytes (Figs. 70—71 and 72).

21. *Abbottina rivularis* (Basilewsky)

This species inhabits the streams of southern Japan. The fishes for this study were collected in two different localities of Osaka Prefecture and in Lake Biwa.

The male diploid number was 50 without exception (Figs. 73—74). The number of chromosomes was found to be identical to those common in related species so far recorded, but in respect of the karyotype this species is strikingly different from them. The chromosome garniture is quite motley, as V- and J-shaped elements with varying length constitute the complement. A careful examination revealed that the majority assume a submedian or median constriction appearing as a V-shape, have a clear constriction near the inner extremely in each. One pair of these J-shaped elements is outstandingly large in size.

The haploid number, 25, was established with extreme clearness in both primary and secondary spermatocyte metaphases (Fig. 75 and 76). Non-telomitic structure of the chromosomes was also proved in these haploid elements.

In fine, the results of the present study seem to make a contribution to the pician cytology in finding a new karyotype which is rather rare in fishes.

22. *Gnathopogon elongatus elongatus* (Temminck et Schlegel)

Being common in the rivers and lakes of Japan, this species is usually caught from June to October. The material was mostly obtained in the vicinity of Sasayama, Hyogo Prefecture.

There are 50 chromosomes in the spermatogonial metaphase (Figs. 77—78). The diploid complement consists mostly of rod-shaped elements, four of them being conspicuously larger.

The primary spermatocyte shows 25 bivalent chromosomes (Fig. 79). There is no element having unusual structure.

23. *Gnathopogon elongatus caerulescens* (SAUVAGE)

The distribution of the present species is restricted to Lake Biwa. The fishes coming under study were collected in various localities of Lake Biwa (Omatsu, Katada, Ogoto and Ōtsu).

The chromosomes of this fish are just the same in number and shape as those of the preceding related species, *G. elongatus elongatus,* because 50 chromosomes were counted in the spermatogonial metaphase (Figs. 80—81). The chromosomes are all simple rod-shaped, and there is no marked distinctive characteristics beyond that of size and length, except that two pairs of larger chromosomes are easily recognizable. The primary spermatocyte contains 25 chromosomes at metaphase (Fig. 82).

24. *Pseudogobio esocinus* (TEMMINCK et SCHLEGEL)

The present species is a medium size food fish and widely distributes in the fresh waters of Honshu, Shikoku, Kyushu and Korea. The material was obtained in the Mukogawa River, Hyogo Prefecture.

The spermatogonial complex shows at metaphase 52 chromosomes which consist of simple rod-shaped elements (Fig. 83). From their apparent characteristics they all seem to be of telomitic nature. The variation in their length is not very remarkable.

The haploid chromosome number of 26 was ascertained through the observations of the primary and secondary spermatocytes (Figs. 84—85). In the first division, they are all ordinary bivalents in structure.

25. *Hemibarbus longirostris* REGAN

This cyprinid occurs in the rivers of Okayama, Hyogo and Nara Prefecture. The materials for the present study were collected in Ichijima, Hyogo Prefecture.

The diploid number observed in the spermatogonial metaphase was determined as 50 (Figs. 86—87). All the chromosomes are of simple rod-type. They also are gradated in respect to length except of the largest pair.

The chromosome counting in the primary spermatocyte metaphase gave 25, among which one is conspicuously large-sized (Figs. 88).

26. *Ctenopharyngodon idellus* (CUVIER et VALENCIENNES)

This fish which occurs commonly in the fresh waters in Manchuria and China ranging to nothern Indo-China, has been successfully introduced to the ponds and lakes of Japan. The testes from a male specimen obtained through the generosity of Mr. G. Fukuda in the Fukuda Fish Breeding Farm, Tottori City, Tottori Prefecture furnished the material for study.

Close investigations of the spermatogonial metaphase revealed that the number of chromosomes was evidently 48 (Figs. 89—90). Since all the composing elements

assume a simple rod-shape showing a slight variation in length, they are undoubtedly of telocentric nature.

Every first metaphase plate under observations showed consistently 24 bivalent chromosomes as a haploid set (Fig. 91).

The external feature of chromosomes of this species is highly different from that of *Cyprinus carpio* (*2n*, 104, Makino 1939), but rather resembles that of *Carassius carassius* (*2n*, 94, Makino 1941).

27. *Moroco steindachneri* (Sauvage)

The present species is one of the fresh water fishes distributing widely throughout Honshu, Shikoku and Kyushu. Formerly, *Phoxinus* was used as the genus name of this form. A number of specimens which were obtained in the vicinity of Sasayama, Hyogo Prefecture constituted the material for study.

The spermatogonial metaphase shows 54 chromosomes (Figs. 92—93). The diploid set contains at least 8 chromosomes which are remarkable for having submedian centromeres. The remaining ones are telocentric in nature, of which one pair is clearly distinguishable from others by their large size. The karyotype of this form furnishes a new example for the Pisces.

The primary spermatocyte shows at metaphase 27 bivalents without exception (Fig. 94). There constantly occurs a ring-shaped bivalent together with four V-shaped ones. The former may be derived from the pair of largest submedian chromosomes.

28. *Moroco percnurus* (Pallas)
(*Phoxinus percnurus sachalinensis*)

The present species distributes through Hokkaido, Saghalien and Siberia. The material was derived from specimens collected in the vicinity of Sapporo.

The Haploid number observed in the primary and secondary spermatocytes was 27 (Figs. 95—96). In general morphology the bivalents are alike to those of the former species. Four V-shaped bivalents and one bivalent are very remarkable in occurrence among the others on account of their prominent shapes.

29. *Zacco platypus* (Temminck et Schlegel)

This is one of the common fresh water game-fishes in middle and south Japan. The specimens for study were secured in the River Ina, Hyogo Prefecture.

The diploid number observed in the spermatogonial metaphase was given to be undoubtedly 48 (Figs. 97—98). All the chromosomes assume the form of a simple rod showing a slight variation of length and there occurs no element which is provided with the V-shape. A pair of members is outstanding by their large size.

The haploid count in the primary spermatocyte metaphase gave 24; the chromosomes are all ordinary bivalents (Fig. 99). The secondary spermatocyte also shows 23 chromosomes in all equatorial plates so far examined (Fig. 100).

30. *Zacco temmincki* (Temminck et Schlegel)

This is a food fish and occurs in the rivers in middle and southern Japan and

Korea. The material consists of testes derived from several specimens collected in Teramae, near Himezi.

The diploid chromosome count made in several spermatogonial metaphases gave 50 in every case (Figs. 101—102). The diploid complement consisted of 46 rod-shaped chromosomes of varying sizes, and the remaining four are distinct V-shaped, being submedian in structure. Among telomitic chromosomes, two chromosoms are very prominent because of their elongated rod-shape, being in size the largest of all.

31. *Opsariichthys uncirostris* (Temminck et Schlegel)

This species inhabits Lake Biwa and its tributary rivers. The material for this study was obtained from several males collected in Ōmimaiko, Lake Biwa.

The general chromosomal condition of this species is somewhat different from what obtained in other species belonging to the Cyprinidea. In spite of the small size of germ cells, the number of chromosomes is considerably high. The diploid number of chromosomes was found to be decidedly 66 (Figs. 103—104). The chromosomes are all of simple rod-type in telocentric nature. A pair of large elements is easily recognizable by their size. The remaining elements are short rods of varying sizes.

Every first metaphase plate under study showed consistently 33 chromosomes (Fig. 105), each being made up of two equivalent components showing an ordinary structure. The same number of chromosomes, 33, was again observed in the secondary spermatocyte (Fig. 106).

32. *Hemigrammocypris rasborella* Fowler

This species occurs in the fresh waters, its distribution extending from middle Honshu southward to Kyushu. The specimens for the present study were secured in the vicinity of Sasayama, Hyogo Prefecture.

Every spermatogonium contains at metaphase 48 well-defined chromosomes (Figs. 107—108). The karyotype of this species closely resemble that of the previous species, *Zacco platypus*: the diploid set consists of two elongated large elements together with the remaining 46 rod-shaped ones with a slight variation of size.

Every metaphase plate of the primary spermatocytes shows 24 elements which are all bivalents with ordinary structure (Fig. 109).

33. *Ishikauia steenackeri* (Sauvage)

This is a food fish occurring in Lake Biwa and its adjoining waters. The present material came from two different localities, Omatsu, Lake Biwa, and the Fukuda Fish Breeding Farm, Tottori city.

Every spermatogonial division shows 48 chromosomes at metaphase (Figs. 110—111). Most chromosomes seem to be rod-shaped, two of which are very outstanding amongst the telomitic group by their prominent large size, and at least two elements are of V-shape of subtelomitic nature.

The haploid number of 24 was ascertained through observations of primary

spermatocytes (Fig. 112).

34. *Barbus semifaciolatus* Günther

This is one of tropical aquarium fishes: its distribution ranges from southern China to India. The material for the study were obtained in the Hankyu Department Store, Osaka.

Every spermatogonial division shows 52 chromosomes at metaphase (Figs. 113—114). The majority of chromosomes are of telomitic nature, but at least four elements are V-shaped. The largest V-pair shows a submedian centromere, while the other pairs have median centromere.

The primary spermatocyte contains at metaphase 26 bivalent chromosomes, of which two bivalents assume the shaped of a V (Fig. 115). They evidently correspond to two pairs of V-shaped chromosomes in the diploid set.

General Ramarks: The Cyprinidae is the pisian family in which the chromosome survey has been made most extensively, since the chromosomes of eight species and two hybrids were investigated by Makino (1939, 1941), Barigozzi (1937), Makino, Ojima and Matsui (1955), and Matsui, Ojima and Nogusa (1951). The present author has added the data from 19 cyprinoid fishes to the above data.

Interesting to note is the fact that there is a considerable difference in the chromosomes of these species, so far as the morphology of chromosome is concerned. There have been known 13 different karyotypes; they are 1) $2n$, 44=2V's+42r's for *Rhodeus*, 2) $2n$, 44=4V's+40r's for *Acheilognathus*, 3) $2n$, 48r's for *Zacco*, *Ctenopharyngodon* and *Hemigrammocypris*, 4) $2n$, 48=2V's+46r's for *Ishikauia*, 5) $2n$, 50r's for *Sarcocheilichthys*, *Hemibarbus* and *Gnathopogon*, 6) $2n$, 50=4V's+46r's for *Acheilognathus*, *Pseudorasbora*, *Pungtungia* and *Zacco*, 7) $2n$, 50=6V's+44J's for *Abbottina* and *Tribolodon*, 8) $2n$, 52r's for *Pseudogobio*, 9) $2n$, 52=4V's+48r's for *Barbus*, 10) $2n$, 54=8V's+46r's for *Moroco*, 11) $2n$, 66r's for *Opsariichthys*, 12) $2n$, 94r's for *Carassius*, 13) $2n$, 104r's for *Cyprinus*. Most of these complements have never been recorded before in any species of fish. Summerizing the above findings, it is evident that each species has its own specific karyotype different from others, irrespective of the fact that whether any two species are closely related or not in their systematic relation.

Working with *Cyprinus carpio specularis*, Barigozzi (1937) has found 19 chromosomes for the male diploid complement. The number of chromosomes reported for this form can not be regarded as conclusive. It seems probable that his results may be erroneous, being induced by inadequate technique.

J. Family Cobitidae

The chromosome survey of this family has hitherto been carried out in the following two species: *Misgurnus anguillicaudatus* and *Barbatula oreas*, by Makino (1941). The chromosomes of two species are to be investigated in this study.

35. *Lefua echigonia* Jordan et Richardson

The loach occurs in the fresh waters of Honshu and Shikoku. It breeds in June.

The specimens used for the study were collected in the vicinity of Sasaayama and Ichijima, Hyogo Prefecture.

The polar view of the spermatogonial metaphase invariably shows 50 distinct chromosomes arranging radially in the equatorial plate (Figs. 116—117). Considered from their external appearance all the chromosomes seems to have a telomitic centromere showing gradual diminution in length.

There are observable 25 bivalents in the primary spermatocyte (Fig. 118). They divide into two equal halves in the first division. The secondary spermatocyte shows again 25 chromosomes of dyad nature (Fig. 119).

36. *Gobitis biwae* JORDAN et SNYDER

This species widely distributed in Honshu, Shikoku, Kyushu, Korea, Manchuria, China and Europe. The material for study were mostly collected in Amagasaki near Osaka and in the vicinity of Sasayama, Hyogo Prefecture.

The germ cells of the present species are considerably larger in size than those of *Lefua echigonia*. The metaphase of the spermatogonial division shows 54 chromosomes, the number being four more than that of the former species (Figs. 120—121). The diploid complement includes four prominent V-shaped elements in two pairs, together with the remaining rod-shaped ones with terminal centromere.

The primary spermatocyte distinctly shows 27 bivalents at metaphase, two of them suggesting to be of atelomitic nature in their structure (Fig. 122).

According to MAKINO (1941), *Misgurnus anguillicaudatus* show the diploid number of 52 consisting of all telomitic elements, while in *Barbatula oreas* it is redaced to 48, but they include two V-shaped atelomitic elements. Thus the numerical relation of chromosomes between the related forms, *Misgurnus* and *Barbatula,* may be explicable as a result of the formation of multiple chromosomes by means of the union of two rods at their inner ends. *Lefua echigonia* and *Cobitis biwae* herein studied show no cytological kinship to the two species mentioned above; the former species has 50 chromosomes which are all telomitic elements, while the latter possesses 54 chromosomes composed of four prominent V-shaped and 50 telomitic ones. There is no similarity between these two species in their chromosomes.

K. Family Siluridae

Cytological investigations of this family were initiated by the present author (1951), who reported the chromosomes of *Parasilurus asotsu.*

37. *Parasilurus asotus* LINNÉ

This is a well-known fish widely distributing in the inland waters of Japan, Korea, Manchuria and China.

The spermatogonium contains 58 well-defined chromosomes in the metaphase plate (Figs. 123—124). The chromosomes are all telomitic rod-shaped, being gradual in length.

The separation of chromosomes of the first division alway takes place synchronously and neither precession nor succession could be observed in any chromosome (Fig. 125). The primary spermatocyte metaphase shows 29 bivalent chromosomes (Fig.

126). There is produced only one kind of the chromosome complex in the resulting secondary spermatocytes. Every metaphase shows without exception 29 dyads (Fig. 127).

Thus it is evident that the chromosome number of this fish is 58 in diploid, and 29 in haploid, and that the male chromosome complex is formulated as 58r's.

L. Family Bagridae

No cytological study of this family have been made by any author prior to the present study.

38. *Pelteobagrus nudiceps* (Sauvage)

This is a voracious fish and inhabits rivers and lakes lying southward from the middle of Japan. The material employed in this study were collected from two localities, Sasayama (Hyogo Prefecture) and Lake Biwa.

The number of spermatogonial chromosomes was decided as $2n$, 56 which are all of rod-type characterized by terminal centromere in each showing gradual diminution in length (Figs. 128—129).

The haploid number was observed to be 28, through observations of primary spermatocytes (Fig. 130). Thus, the chromosome formula of this species is given by 56r's. The chromosome number of the present species is two less than that of the former species (Siluridae). However, there is a considerable similarlity to their kinship in morphological taxonomy.

Order Anguillida

The cytological study of this order has been carried out in only one species of the Anguillidae by Rodolico (1933). The present paper described the chromosomes of one species of the Echelidae and two species of the Muraenidae.

M. Family Echelidae

The chromosomes of only one species of the family were investigated here.

39. *Echelus uropterus* (Temminck et Schlegel)

In Japan, this species is rarely found in the waters southward Honshu. The specimens from which the present material was derived were obtained by the use of a drag-net in Owashi, Wakayama Prefecture.

The chromosome counts were made in this study in a considerable number of spermatogonial metaphases, showing that the diploid number was 50 in every cell under study (Figs. 131—132). Four chromosomes are very prominent because of the remarkable J-shape with a submedian centromere. They evidently constitute a homologous pair. The remaining chromosomes seem to be rod-shaped from their external appearance, varying in size. The haploid number of chromosomes was found as 25 through observations of the primary spermatocytes (Fig. 133).

In conclusion, the chromosome formula of this species is given as 4J's+46r's.

N. Family Muraenidae

The present study deals with the chromosomes of two species of this family.

40. *Muraena pardalis* Temminck et Schlegel

This is a species of ells occurring southward from the middle Japan to East Indias. The materials employed consisted of the testes obtained from two adult

specimens. They are collected in Tanabe, Wakayama Prefecture.

The diploid complex is remarkable not only for a comparatively low number, but also for the fact that several pairs of the chromosomes are V-shaped. The diploid number of chromosomes, as counted in the spermatogonial cells, was determined to be constantly 40 (Figs. 134—135), 14 of which are V-shaped elements with the submedian centromere and the remaining 26 are rod-shaped ones of telocentric nature.

Every primary spermatocyte metaphase shows 20 chromosomes of bivalent structure, seven elements of which assume the shape of a V (Fig. 136). They evidently correspond to seven pairs of V-shaped chromosomes in the diploid set.

41. *Gymnothorax kidako* (Temminck et Schlegel)

This moray ell distributes southward from middle part of the country on the Pacific coast. It lives among the rocks along the coast, sometimes in the tide pools. The specimens collected in Fukura, Awaji Island constituted the material for study.

In magnitude the cell of this species is largest among those of the teleost fishes so far studies by the present author. The diploid number of the chromosomes was observed to be 42 in every spermatogonial metaphase plate (Figs. 137—138). The number is two more than that of the former species. Among the 42 chromosomes, six pairs are very prominent in having remarkable atelomitic structures of submedian and subterminal centromere. The members of the remaining fifteen pairs are regarded as telomitic in their structure.

The haploid chromosomes was ascertained to be 21 in the primary spermatocyte (Figs. 139—140). It is then evident that the chromosome formula of this species is 12V's+30r's=42 for the male diploid group.

From the above results, it is evident that the chromosomes of this family are characterized by a relatively low number as compared with those of other families. The numerical and morphological differences of chromosomes between *Muraena* and *Gymnothorax* may be accounted for on the basis of the formation of multiple chromosomes by means of centric fusion. Noticeable is the fact that the karyotypes of these two forms are of rarely occurring among teleosts.

Order Percida

Knowledge on the chromosomes of this order is limited to four families; two species of the Anabantidae, one species of the Atherinidae, two species of the Percidae and one species of the Pholidae have cytologically been studies by Moenkhaus (1904), Turner (1919), Bennington (1936), Makino (1937) and Svärdson and Wickbom (1939, 1942). The present author has had a chance to investigate the chromosomes of each one species in the following families, Serranidae, Sillaginidae, Parapercidae, Callionymidae and Labridae, and eight species of the Gobiidae.

O. Family Serranidae

So far as the author is aware, no reference is accessible to the literature on the chromosomes of the Serranidae.

42. *Coreoperca kawamebari* (Temmink et Schlegel)

This is a common fresh-water fish in the rivers of south Japan. The present material was based on testes obtained from the male specimens which were collected in Sasayama, Hyogo Prefecture.

The diploid number of chromosomes observed in the spermatogonial metaphase plate of this species is 48 without exception (Figs. 141—142). All the elements are uniformly terminal in their fibre attachment. It seems impossible to identify the homologous pairs, because all the chromosomes show slight size difference.

The chromosome counts made in the primary spermatocyte division consistently gave the reduced number of 24 (Fig. 143).

It is evident that the present species possesses 48 chromosomes in diploid and 24 in haploid. The male chromosome complex is represented by the formula of 48r's.

P. Family Sillaginidae

The chromosomes of *Sillago sihama* were observed by the present author (1951) as given below.

43. *Sillago sihama* (Forskal)

Sillago sihama ranges in distribution from Hokkaido southward to the wide area of Indo-Pacific including as far as Red Sea. It frequents shallow sandy-bottom of small bays, and spawns in August and September. The material consists of the testes from a several specimens captured by fishing in the Osaka Bay.

The number of spermatogonial chromosomes is 48 (Figs. 144—145). The individual chromosomes bears no characteristic appearance beyond that of length. Considered from the external feature, the chromosomes seem to be of simple rod-type, and show a slight variation in size.

The metaphase of the primary spermatocyte was found to exhibit 24 ordinary bivalents (Figs. 147—148). The separation of the sister chromosomes is quite synchronous in every elements (Figs. 146, 149). Nothing particular is found in the mode of the primary spermatocyte division. Every equatorial plate of the secondary spermatocytes show 24 chromosomes (Fig. 150).

The present species shows 48 chromosomes in diploid and 24 in haploid, with the chromoseme formula of 48r's.

Q. Family Parapercidae

In the following, the chromesomes of *Cilias pulchella* are to be reported.

44. *Cilias pulchella* (Temminck et Schlegel)

Living in the small bays, this food fish ranges in distribution from middle Japan southward to South China Sea. The present material was derived from the testes of male specimens obtained in Iwaya, Awaji Island, Hyogo Prefecture.

The diploid number of chromosomes was observed to be 48 in the spermatogonial metaphase (Figs. 151—152). The diploid members seem to be of atelomitic nature of varying size. The elememts forming the largest pair are prominent among the others. The karyotype as found in this species is quite unique among fishes so far reported.

The haploid number of chromosomes was clearly established as 24 in the primary spermatocyte (Figs. 153—154).

The chromosome number of this species is 48 in diploid and 24 in haploid, and the chromosome formula is represented by 48V's.

R. Family Callionymidae

No species has been studied cytologically in this family. In the present study the chromosomes of *Callionymus richardsoni* were observed.

45. *Callionymus richardsoni* Bleeker

The species is common southward from middle Japan to southern Korea and South China Sea. The fishes for the present investigation were collected from various parts in the Osaka bay.

The diploid number was determined to be constantly 38 in the spermatogonial metaphase (Figs. 155—156). The chromosomes are all telomitic rod-shaped, some straight, and some more or less curved. All the chromosomes can be arranged into 18 homologous pairs and no unpaired elements can be identified, as is obviously demonstrated in the serial arrangment of chromosomes (Fig. 157).

The chromosome counts in the primary spermatocyte consistently gave the reduced number of 19 (n) (Figs. 158—159). There is no evidence for the presence of any particular chromosome in behavior or in morphology.

S. Family Gobiidae

The chromosomes of the Gobiidae have been studied by the present author (1950, 1955, 1957) in nine species as give below.

46. *Mogrunda obscura* (Temminck et Schlegel)

Mogrunda obscura is a small teleost of the Gobiidae, very common in fresh waters in southern Japan. The fishes for material were collected in various streams in the vicinities of Amagasaki and Sasayama, Hyogo Prefecture.

The present author has found definite evidence regarding the sex-chromosomes showing a clear-cut XY mechanism in this species, and therefore the descriptions may be made in some detail on the behavior of the XY-chromosomes.

Behavior of the X and Y during the growth period: The history of the chromatin nucleolus up to transformation into the XY-complex during the growth period will be traced. The nucleus of the growing spermatocyte following the last spermatogonial telophase shows two distinct nucleoli. Considered from their behavior in the course of the growth period as given below, one of them is to be regarded as the chromatin nucleolus, and the other the true nucleolus (plasmosome). They are clearly distinguishable from one another: the chromatin nucleolus is nearly oval in shape and shows an intense affinity to haematoxylin, while the plasmosome is spherical and stained uniformely, but it is not compact in texture. Through the early leptotene stage, the chromatin nucleolus shows a gradual elongation, with a slight bending at a constriction near its extremity (Fig. 162). It grows longer step by step into an elongated body with a sharply defined contour. Then, a minute element appears at the terminal

part of the elongated body. In this condition the elongated body exhibits a distinction into the two components, of which the longer part is to correspond to the X-element and the minute one to the Y (Figs. 163—164). With the passage of the stage from leptotene to pachytene, the X- and Y-element become gradually shortened by condensation (Figs. 165—166). In the diplotene stage they are converted into the form characteristic to the XY-complex with a definite outline consisting of a large X and a small Y in end-to-end association. The XY-complex persists without any change of its shape during the latter part of the growth period through diakinesis (Fig. 167) to metaphase of the first division. The XY-complex is observable with a clear distinction from the autosomal elements due to its characteristic structure. Thus, the X- and Y-elements were traced each as heteropycnotic bodies in the nucleus of the growing spermatocyte from early leptotene to diakinesis.

The primary and secondary spermatocytes: In the polar view of the primary spermatocyte metaphase the bivalent chromosomes appear in distinct clearness each showing a definite outline. This makes the chromosome count very easy. Every metaphase plate is provided constantly with 31 chromosomes as the haploid complex, which consists of 30 autosomal bivalents with ordinary structure of various sizes, and an XY-bivalent which is very prominent due to its heteromorphic configuration (Figs. 168—169). The XY-bivalent always lies in a peripheral area of the equatorial plate. It consists of an elongated rod-shaped X-element connected at its extremely with the small-sized Y.

In the lateral aspect, the X seems to correspond in its length to one of the larger elements, while the Y corresponds to that of the smallest one. In relative magnitude the X element seems to be about twice the size of the Y (Figs. 170—172). The mode of conjugation between the X and Y is very characteristic; in conjugation the X-element always stands nearly vertical to the equatorial plate, holding its long axis parallel to the spindle axis, while the Y lies horizontally in the equatorial plate with its long axis lying parallel to the latter, connecting end-to-end with the X. The spindle fibers possibly attach to the free end of the X and to the inner proximity of the Y where the X comes in contact. A similar type of conjugation of the X- and Y-elements was found to occur in some bovine mammals by Makino (1944).

In the first division the X-element segregates from the Y without exception and they migrate to the opposite poles, as do the autosomal bivalents. The first division results in the production of two sorts of secondary spermatocytes; one half of the secondary spermatocyte carry the X and the other half of them the Y.

Every metaphase plate of the secondary spermatocyte shows 31 chromosomes of dyad nature (Figs. 173—174). Theoretically there should occur two kinds of secondary spermatocytes with respects to the X and Y, but the distinction of the two sorts is quite difficult, because the X and Y bear no characteristic feature to render them distinguishable from the autosomal elements.

The spermatogonium: The diploid number of chromosomes established in the

study of the spermatogonia was found to be constantly 62 (Figs. 160—161). The chromosomes are all of simple rod-type, being telomitic in nature. It is impossible to identify the homologous pairs, on account of the high number of chromosomes with slight differences. Thus, picking out the X- and Y-chromosomes by identification of the homologous pairs of chromosomes was impossible. But, judging from the data obtained from the observations in the growing period and in the first meiotic division, it is natural to consider that the even number of spermatogonial chromosomes suggests the occurrence of the XY sex-chromosomes.

In conclusion, the diploid number of chromosomes of the present species was 62 in the spermatogonium and the haploid number of 31 was observed in both primary and secondary spermatocytes. All the chromosomes are characterized by a simple rod-type of telomitic nature. Remarkable is the occurrence of the sex-chromosomes of the XY-mechanism found in this species. The present report is the first to record the cytological evidence of male heterogamety in fishes.

47. *Tridentiger obscurus* Temminck et Schlegel

Preferring brackish and fresh water, this species lives near the estuaries or in the lagoons of Japan and Korea. Testicular tissues from a numerous specimens obtained in Amagasaki, near Osaka, furnished the material.

The spermatogonial metaphase plate contains 44 chromosomes (Figs. 175—176). The diploid complement includes two pairs of small elements which are of conspicuously smaller in size in comparison with the other chromosomes, and this condition is again observed in the primary and secondary spermatocytes.

The first spermatocyte metaphase shows 22 bivalents of various sizes (Figs- 177—178). One of them is a conspicuous small chromosome in a striking contrast to others. Occasionally, the author observed 23 chromosomes in this stage, due to the inclusion of two univalent chromosomes (Fig. 179). Thus, it is evident that the complement showing 23 chromosomes consists of 21 bivalents and 2 univalents. On a close examination of these cells, the univalent were distinguishable from the bivalent in their shape and structure. The cell with the univalent chromosomes have appeared at first metaphase in proportion of 2.9 per cent. There are also counted 22 chromosomes in the metaphase plate of the secondary spermatocytes.

48. *Gobius similis* (Gill)

This is one of the most common fresh water fishes in Japan. The specimens for the present study were obtained in Sasayama, Hyogo Prefecture.

Every spermatogonial plate constantly showed 44 chromosomes (Figs. 180—181). The chromosomes seem to be of a telomitic type, because all the composing elements appear in the form of a short rod having a slight fluctuation in length, though it is difficult to say whether they are acrocentric or telocentric in nature.

The chromosome count in the primary spermatocyte metaphases gave 22 as the reduced number (Fig. 182).

49. *Gobius abei* (Jordan et Snyder)

Preferring brackish water, this species abounds near the estuaries or ascends of the rivers in middle and southern Japan. The specimens for study were secured in Oomuta, Kyushu.

The diploid number observed in the spermatogonium was clearly 46 (Figs. 183—184), being two more than that of the former species. The chromosomes appear to be of a simple telomitic type in morphology, since all elements assume a short rod-shaped appearance showing a slight variation in length, two of them being conspicuously smaller in striking contrast to others.

The haploid chromosome number of 23 was determined through observations of primary spermatocytes (Figs. 185).

50. *Acanthogobius flavimanus* (Temminck et Schlegel)

This fish ranges in distribution from southern Hokkaido southward to Kyushu. The specimens for study were collected in Osaka Bay.

Every spermatogonial metaphase contains 44 chromosomes (Figs. 186—187). Each of the chromosomes have a terminal centromere, showing a well-graded series in length, with the exception of the two pairs which are slightly remarkable for their minute size.

The haploid number was obtained as 22 from observations of the primary and secondary spermatocyte divisions (Figs. 188, 189).

51. *Chaenogobius urotaenia* (Hilgendorf)

This is also of very common freshwater fishes in Japan. The study was based on the material obtained in Koyama, Tottori Prefecture.

Spermatogonial metaphase showed always 44 chromosomes (Figs. 190—191). The diploid complement consists of simple rod-shaped elements of various length, a single pair of which are extremely small in contrast to others.

The primary spermatocyte metaphase always shows 22 bivalents containing a small-sized one (Fig. 192).

52. *Chaenogobius isaza* (Tanaka)

The isaza goby lives at the depth of 17 or 18m in Lake Biwa, Shiga Prefecture and in the rivers pouring into the lake. The specimens for observations were obtained in Katada, Lake Biwa.

The diploid number observed in spermatogonial metaphase was given to be 46, two more than that of the former species (Figs. 193—194). The chromosomes are all of a simple rod-type. This species is characterized by having two pairs of small-sized chromosomes in its complex.

The primary spermatocyte metaphase shows 23 bivalents, of which two are strikingly small in size (Fig. 195).

53. *Boleophthalmus pectinirostris* (Osbeck)

This species is abundant in Ariake Bay, Kyushu, Japan. The material on which the study was based came from Yanagawa, Kyushu.

In the spermatogonial metaphase there are observable 46 distinct chromosomes

(Figs. 196—197). The chromosomes are all rod-shaped in appearance. They show a gradual diminution in length, two pairs being extremely small.

The metaphase plate of the primary spermatocyte contains 23 bivalent chromosomes (Fig. 198).

54. *Periophthalmus cantonensis* (Osbeck)

This fish ranges in distribution along the coast of the southern part of Japan, hopping about on the mud-flats in ebb-tide. The present material was collected in Oomuta, Kyushu.

The diploid number of chromosomes was found to be 46 in the spermatogonium, and the haploid number to be 23 in the primary spermatocyte (Figs. 199—201). The diploid complex consists of rod-shaped elements with a variation of length. It is a noticeable fact that this species is characterized by containing three pairs of remarkably small-sized chromosomes.

General Remarks: The chromosome study of the Family Gobiidae has been carried out in nine species by the present author (Nogusa 1950, 1955, 1957), with special regard to the chromosomal relationship in closely related species. It is remarkable that the chromosomes of *Mogrunda obscura* are considerably different from the other eight species, showing 62 in $2n$. The XY-chromosomes were clearly distinguishable from the autosomes during a period from the meiotic prophase to metaphase of the primary spermatocyte. This is the first to record the occurrence of the XY-mechanism in fishes with indisputable clearness. The chromosome numbers and chromosomal formulae of eight species of the Gobiidae here under consideration are as shown in Table 6.

Table 6. Chromosome numbers in the Gobiidae studied by the author

Species	Chrom. number		Chrom. formula
	$2n$	n	
Tridentiger obscurus	44	22	42r's + 1m's
Acanthogobius flavimanus	44	22	42r's + 2m's
Gobius similis	44	22	44r's
Gobius abei	46	23	44r's + 2m's
Chaenogobius urotaenia	44	22	42r's + 2m's
Chaenogobius isaza	46	23	44r's + 2m's
Boleophthalmus pectinirostris	46	23	40r's + 6m's
Periophthalmus cantonensis	46	23	42r's + 4m's

r: rod-shaped chromosome. m: minute chromosome

Comparison of the chromosomes made it clear that the karyotypes of the eight species here under study are closely related with each other, though there is a variation of the chromosome number from 44 to 46. Further, every one is characterized

by possessing a definite number of the minute chromosomes which is tentatively referred to as m-chromosome, with the exception of *Gobius similis* which has no such element.

Tridentiger obscurus, Achanthogobius flavimanus, Gobius similis and *Chaenogobius urotaenia* were found to possess 44 chromosomes in diploid, all elements being of a telomitic type with gradual difference of length. It was pointed out that the m-chromosome exhibits a size variation by species; the m-chromosomes of *Achanthogobius flavimanus* and *Chaenogobius urotaenia* are slightly larger than those of *Tridentiger obscurus*. According to the view of Wilson (1928), the m-chromosome generally contains no important gene.

The chromosome of *Gobius abei* are 46 in diploid, consisting of 44 simple rod-shaped chromosomes and two m-chromosomes, whereas in *Gobius similis* the diploid number is 44, containing no element corresponding to the m-chromosome. The chromosome number of *G. similis* is two less than that of *G. abei*. Thus, the numerical and morphological relationship of chromosomes between two species of *Gobius* may be explained on the basis of the present and absence of the m-chromosome.

The similar relationship is found to occur between *Chaenogobius urotaenia* and *Chaenogobius isaza*. If the m-pair of *C. isaza* would disappear, the induced number of chromosomes becomes 44 in total, that is the number for *C. urotaenia*.

Boleophthalmus pectinirostris possesses three pairs of m-chromosomes, while the related species, *Periophthalmus cantonensis*, has two pairs of such elements. The only difference between the two forms deals with the number of the m-chromosomes. According to the view that the m-chromosome carries no important genes, it is probable that these two species are akin to each other in both taxonomical relationship.

It has been shown by Oguma (1930) that the reduction of the chromosome number in certain species of dragonflies is due to an absence of a pair of the minute chromosome as referred to as the m-chromosome. Several such instances have been reported to occur in some other animals showing that gradual size-reduction of the m-chromosome and their final disappearance occur between related species; the examples are demonstrated in dragonflies by Oguma (1930), Oguma and Asana (1932) and Kichijo (1939), in grasshoppers by Momma (1943), in lizards by Oguma (1934) and Nogusa (1953b), and in murine rodents by Makino (1941, 1942, 1943). It is then apparent that the disappearance of the m-chromosome seems to play an significant role in the change of chromosome number from species to species in animals.

T. Family Labridae

Previously, Pinney (1918) has reported on the somatic chromosomes of *Ctenolabrus adspersus* of this family. An additional data obtained from a Japanese species will be added here.

55. *Pseudolabrus japonicus* (Houttuyn)

In Japan this laboroid species occurs in the warm waters southward from middle Honshu. The species obtained in Iwaya, Awaji Island, furnished the material.

Careful counting of several metaphase plates of spermatogonia gave 46 as the diploid number (Figs. 202—203). The diploid complement is very prominent in containing two conspicuous large V-shaped elements which constitute a homologous pair. The remaining 40 elements are found to be of simple rod-type, ranging from long rods to very short ones.

The haploid number was established as 23 from observations of the primary spermatocyte (Fig. 204).

Thus, the male diploid complement of this fish is given a formula of 2V's+44r's.

Order Tetraodontida

This order has never been dealt with cytologically by any author. In this study, three species of the Monacanthidae were cytologically investigated.

U. Family Monacanthidae (Aluteridae)

In the present paper are reported the chromosome of the following three file fishes: *Stephanolepis cirrhifer, Novodon modestus* and *Rudarius ercodes.*

56. *Stephanolepis cirrhifer* (Temminck et Schlegel)

This is the commonest species of the Japanese file fishes. The material used for present study was obtained from several males collected in Iwaya, Awaji Island.

The present form shows the lowest number of chromosomes in the three species under study. Every spermatogonial plate showed 34 without exception (Figs. 205—206). All the members take the form of rods, being telocentric in appearance.

The haploid number of chromosomes was difinitely established as 17 by the observation of the primary spermatocytes (Fig. 207). The secondary spermatocytes showed again 17 chromosomes of dyad nature (Fig. 208).

57. *Rudarius ercodes* Jordan et Fowler

This species is very common in the shallow bays in southern Japan. The material for the study was collected in Maizuru Bay, Kyoto Prefecture.

The diploid number of chromosomes of this species is two more than that of the former species, *Stephanolepis cirrhifer,* since the spermatogonial metaphase showed 36 chromosomes (Figs. 209—210). The chromosomes are all of the simple rod-type, varying slightly in length.

The haploid number of chromosomes was determined as 18 in the primary spermatocyte (Fig. 211). Thus, the chromosome formula of this species is given as 36r's (2*n*).

58. *Novodon modestus* (Günther)

This is a common species on the coasts of Japan from Kyushu and southern Korea. The present material was derived from male specimens obtained in Iwaya, Awaji Island.

There are 40 chromosomes in the spermatogonial metaphase (Figs. 212—213). As usual, all the chromosomes are very remarkable in structure.

The primary spermatocyte contains 20 bivalents in every metaphase plate (Fig. 214).

Generally speaking, the chromosomes of this family are characterized by the relatively low number as compared with those of other families here concerned. The above three species are nearly alike in their chromosomal constitution, since the chromosomes are all of a simple rod-type and probably telocentric in nature in every species.

Order Cottina

The chromosomes have hitherto been known from one species of the Cottidae (Hann 1927), and one species of the Hexagrammidae (Makino 1937) in the order Cottina. Here the present author has chanced to study the chromosomes of each one species of the Synanceiidae, Hexagrammidae and Cottidae.

V. Family Synanceiidae

Previously the cytological study has never been made by any author in this family.

59. *Inimicus japonicus* (Cuvier et Valenciennes)

Inimicus japonicus commonly occurs in the waters extending from middle Honshu southward to Kyushu and southern Korea. Testicular material from two specimens obtained in Iwaya, Awaji Island, provided the samples for study.

After careful counting it became evident that the diploid number of chromosomes in spermatogonia is 50 (Figs. 215—216). From their superficial characteristics they all seem to be of telomitic nature. The variation in their length is not very remarkable.

The haploid number, 25, was clearly observed in the primary spermatocyte (Fig. 217). The chromosomes found in the secondary spermatocyte are all telomitic rod-shaped with tapering ends (Fig. 228).

In addition to the above basic feature, the samples from a specimen showed that the first meitotic metaphases contained a certain number of univalent chromosomes. The details on this matter will be described in the following.

Occurrence of the univalent chromosomes: While working with slides derived from an individual of this species, the author found a number of metaphase plates of the primary spermatocytes which showed chromosome numbers higher than the basic number of 25; they showed the haploid numbers of 26, 27, and occasionally 28. After closer examination, it became evident that such aberrant numbers were due to the occurrence of univalent chromosomes. Thus, the complement showing 26 chromosomes consists of 24 bivalents and 2 univalents, and that showing 27 has the complex containing 23 bivalents and 4 univalents, and so on (Figs. 218—220). The numerical variations in the haploid complement was found in a single individual among three specimens studied here. The chromosome constitution in each complement is as shown in Table 7: of 150 first metaphase plates here observed the univalent frequency ranged from 2 to 6. The complement having no univalent chromosomes was found at 63.33 per cent.

The univalent was clearly distinguishable from bivalents in their shape and

Table 7. Chromosome constitution in the first spermatocyte metaphase plate

Haploid	25	26	27	28	Total
Complex	25II	24II+2I	23II+4I	22II+6I	
No. of cells obv.	95	30	19	6	150
Frequency	63.33%	20.00%	12.67%	4.00%	100%

II: bivalent chromosome. I: univalent chromosome.

structure. The univalents, especially in lateral view, are very clearly observable due to their precocious migration to one of the poles. At metaphase, the dumb-bell shaped bivalents arrange themselves on the equatorial plate forming a single plane, while the univalents irregularly scattered on the equatorial plate (Figs. 221—224). It is highly probable that, as a consequence of the failure of pairing during meiosis, one or more pairs of chromosomes appear as univalents. The cause which induce the failure of pairing in chromosomes remains unknown.

The behavior of univalents at metaphase and anaphase of the first meiotic division is interesting. The mode of migration of the univalents to the poles at the first anaphase varies by cells; the data of observations are as shown in Table 8.

Table 8. Mode of migration of univalents in first meiosis

No. of univalents	2		4			6				
Mode of separation	1—1	2—0	4—0	3—1	2—2	6—0	5—1	4—2	3—3	Total
No. of cells obv.	21	35	12	15	7	1	3	7	4	105

At anaphase and telophase of the first meiosis, the univalents which run ahead other autosomes formed frequently small extranuclei (Fig. 225). It seems very likely that such abnormal germ cells do not preceed beyond the early prophase stage of the second meiosis and degenerate without forming the metaphase spindle.

In the secondary spermatocyte metaphase, however, five classes of chromosome numbers were observed; they were 24, 25 and 26, rarely 23 and 27 in number (Figs. 226—230). They all divide synchronously at anaphase. From the above results, five kinds of spermatids will be produced, each having 23, 24, 25, 26 and 27 chromosomes. The testicular cysts show spermatids in various stages of metamorphosis, together with maturing spermatozoa. The question remains unsolved as to whether these secondary spermatocytes with aberrant chromosome numbers develop into functional spermatozoa, or not.

It is evident from the above results that the normal or basic number of chromosomes of the present species is 50 in diploid and 25 in haploid. The chromosome complex is typically isomorphic, every element being uniformly short and rod-shaped. Occasional occurrence of cells with univalent chromosomes at first metaphase was observed in an individual. The cells with univalents seem to occur in a rather high frequency.

W. Family Hexagrammidae

The chromosomes of *Hexagrammos octogrammus* were studied by Makino (1937). The present paper describes the chromosomes of a related species, *Hexagrammos otakii*.

60. *Hexagrammos otakii* Jordan et Starks

This is the commonest species of hexagrammid fishes in Japan which ranges in distribution from southern Hokkaido southward to Kyushu and southern Korea. The present material consisted of testes obtained from a male specimen in Akashi, near Kobe.

The diploid number of chromosomes was determined to be 48 in the spermatogonial metaphase (Figs. 231—232). The diploid complement consists of rod shaped elements of various length.

The number of haploid chromosomes was 24, based on the observation of the primary spermatocytes (Fig. 233).

The general configuration of the chromosomes of *Hexagrammos otakii* seems to be identical with that of *H. octogrammus*, either in the number of chromosomes or in some morphological characteristics, in fair correspondence to their taxonomical kinship.

X. Family Cottidae

Referring to the literature, the chromosomes of one species, *Cottus bairdii*, have already been reported in this family by Hann (1927). The present author has established the occurrence of male heterogamety in the related species *Cottus pollux*.

61. *Cottus pollux* Günther

Cottus pollux is one of the common fresh water teleosts belonging to the Cottidae. They are widely distributed through Japan. The specimens for this study were collected in the vicinity of Sasayama, Hyogo Prefecture.

Behavior of the X and Y during the growth period: Through the stages of the growth period, the primary spermatocytes show a definite chromatic body in each nucleus. Apparently the body is easily distinguished from the nucleolar element on account of the intense affinity to haematoxylin and of the Feulgen positive reaction. Based on the staining reaction of these bodies, it is evident that the former is the heteropycnotic sex-element, while the latter is the true nucleolus (plasmosome). The heteropycnotic sex-element is found free in the nucleus without associating with the true nucleolus. The nucleus at early leptotene shows the heterochromatic sex-element which lies always along the inner side of the nuclear membrane, in contrast to the true nucleolus which occurs in the interior of the nuclear vesicle (Fig. 236). At late leptotene, the sex-element is recognizable as a heart- or V-shaped body, showing a distinction into two components consisting of the X and Y elements in an end-to-end association (Figs. 237—238). The plasmosome nucleolus becomes invisible during the period through late leptotene to zygotene. The heteropycnotic XY-bivalent is very striking in occurrence in the pachytene nucleus (Figs. 239—240). Through diplotene to diakinesis, the XY-body is easily distinguished from the autosome bivalents by its

peculiar configuration.

The primary and secondary spermatocytes: Every primary spermatocyte contains at metaphase 24 distinct chromosomes in haploid. Closer examination has revealed that the haploid complex consists of 23 autosomal bivalents and a heteromorphic bivalent provided with the X- and Y-element (Fig. 241). The latter always occupies a peripheral position in the equatorial arrangement. The structural configuration of the XY-complex is well observed in the side view of the spindle (Fig. 242): the autosomal bivalents are all dumb-bell shaped, lying vertical to the equatorial plate, while the XY-complex is distinctly characterized by an open V-shaped form. The X-element conjugates with the Y by means of an end-to-end association. The size difference existing between the X- and Y-element is very small; the X-element seems to be slightly, but visibly, larger than the Y.

As a result of the segregation of the X and Y in the first division, there are produced two kinds of secondary spermatocytes with regard to the distribution of the X and Y. Every metaphase of the secondary spermatocytes always shows 24 chromosomes (Fig. 243). The author has failed to identify the X- and Y-element in the complement, on account of the slight size-difference between the X and Y.

The spermatogonium; Careful counting of several metaphase plates of the spermatogonia gave 48 as the diploid number furnishing corroborative evidence for the haploid number of 24 (Figs. 234—235). The diploid chromosomes are of simple rod-type with slight variations in length, one pair of which are extremely small. There is no evidence for the occurrence of a metacentric V-shaped element. Judging from the data obtained in the meiotic phases, it is apparent that the spermatogonial complement includes the X- and Y-element. The identification of the X- and Y-chromosome, however, seems to be practically difficult, due to the prevalence of the chromosomes of similar size, and to the slight size-difference between the X and Y.

From the above results the XY sex-determining mechanism was established in male germ cells of *Cottus pollux*, a species of the Cottidae. The diploid number was 48 in the spermatogonium and the haploid number of 24 was found in both the primary and secondary spermatocytes. During the growth period, the X- and Y-elements were traced each as a heteropycnotic body in the primary spermatocytes. The sex-chromosomes are represented by two rod-shaped ones of larger size, and slightly differ in length. The XY-complex is well recognizable in the side view of the first meiotic spindle.

Order Pleuronectidae

The cytological study of this order has remained untouched by any author. The present author was successful to investigate the chromosomes of three species which include one species of the Bothidae and two species of the Pleuronectidae.

Y. Family Bothidae

In this paper, the chromosomes of *Paralichthys olivaceus* are to be described.

62. *Paralichthys olivaceus* (Temminck et Schlegel)

This is one of food fishes: they widely ranges in distribution from Japan to Hongkong. The present material came from two different localities, Shodo Island, Inland of Japan, and Osaka Bay.

The diploid number of chromosomes was observed to be 46 in the spermatogonial metaphase (Fig. 244). All of the diploid elements seem to be telocentric in structure.

The primary spermatocyte metaphase showed the haploid number of 23 (Fig. 245).

Z. Family Pleuronectidae

The chromosomes of two species have been dealt with in the following.

63. *Limanda yokohamae* (Günther)

The species is common in Japan and the coasts of Asiatic continent. The specimens used as material were collected in Osaka Bay.

The spermatogonial metaphase shows 48 chromosomes (Figs. 246—247). The chromosomes are all rod-shaped of various sizes, ranging from elongated rods to short rod-like ones.

The primary spermatocyte in every metaphase plate contains 24 bivalents (Fig. 248).

64. *Kareius bicoloratus* (Basilewsky)

This species occurs in the waters of Japan and Asia. Several specimens collected in Akashi, near Kobe, in October and November 1956 provided the material.

The diploid number of chromosomes was the same as that of the former species being 48 in the spermatogonium (Figs. 249—250).

The haploid number of chromosomes was determined as 24 in the primary spermatocytes (Fig. 251).

From the above observations, it is clear that two species of the Pleuronectidae. *Limanda yokohamae* and *Kareius bicoloratus*, uniformly possess the diploid number of 48 which consist of simple rod-shaped chromosomes. Morphological comparison of chromosomes reveals that there is no essential difference in the chromosomes of the two species, not only in number but also in other morphological features.

Discussion

The taxonomy, variation and distribution of animals have been dealt with by a number of taxonomists and ecologists. On the other hand, chromosome cytology has made considerable contributions to animal taxonomy. Cytological data serve as useful and basic criteria for the diagnosis of species and for understanding the mechanism of the evolution of organisms, since changes in chromosomes play an significant role in the formation of species (White 1954). In view of the above consideration, the cytological feature of the Pisces is of great interest in connection with the evolution of vertebrates. At the present time, the number of described species of fishes is believed to be somewhat over 13,000 (Suyehiro 1951). Of this total, about 130 (0.01%) have been studied by chromosome cytologists to more or less degree, as re-

ferred to in the monograph published by MAKINO (1956). The pioneer workers dealt chiefly with the spermatogenesis or egg development in the Elasmobranchs and Cyclosomes, and therefore such early work of doubtful accuracy is omitted from consideration in this paper. Thus it is evident that basic data for understanding the evolution of the mechanism of heredity in fishes are still very incomplete. Seriously analytical and experimental studies have been carried out on only a very small part of the species mentioned above.

In the following discussion, the author will consider the cytological mechanism in relation to species formation and evolution of fishes by reference to the data so far accumulated by cytologists, specially with reference to the cyto-taxonomic differences which exist between related species.

1. Cytological relation between the Prochordata and lower vertebrates

WHITE (1954) has stated that all evolutionary changes have had their origin in the chromosomes, and therefore the behavior of chromosomes during phylogeny is of great importance in relation to the evolution of the organism. The phylogenetic relationship of the Prochordata to the Vertebrata has long been a matter of particular interest in systematic taxonomy. Based on the karyological data, some phylogenetic considerations of the lancelet will be offered below.

The evolutional relationship of the lancelet (*Amphioxus*) to such lower vertebrates as cyclostomes and fishes is to be considered from the karyological view point. As given in the foregoing pages, the chromosomes of the lancelet are isomorphic in complex, i. e., each chromosome is of simple rod-type with a taper end and each one shows no remarkable size-difference, except for the X- and Y-elements which have their own characteristic shape and size. In general appearance the chromosomes of the lancelet rather resemble those of various teleost fishes (WINGE 1923, GEISER 1924, IRIKI 1932, RALSTON 1934, FRIEDMAN & GORDON 1934, MAKINO 1934, 1935, 1937, 1939, 1941a, 1941b, BENNINGTON 1936, NOGUSA 1950, 1951a, 1951b, 1955, 1957a, 1957b)

The present study has dealt with the chromosomes of three members of the Cyclostomata, *Entatretus burgeri* and *Entatretus okinoseanus* and *Entoshenus reissneri*. It was found that the chromosome number was 48 in *Entatretus burgeri*, 46 in *Entatretus okinoseanus*, and 94—96 in *Entosphenus reissneri*. The chromosomes are all of simple rod-type in these three forms. Whatever the phylogenetic relation may be, the observed facts seem to indicate that the lancelet shows a considerable karyological kinship to some of the cyclostomes. It can be said therefore that there is evidence indicating a karyological similarity among the lancelet, some teleosts and cyclostomes. In the light of these facts, it seems probable that there certainly does exist a phylogenetic relationship between the lancelet and lower vertebrates.

A similar relationship is also suggested to occur with respect to the sex-chromosome mechanism The XY-mechanism was established as occurring in two forms of teleost fishes by the present author (NOGUSA 1954, 1955), one in *Mogrunda obscura* (Gobiidae) and the other in *Cottus pollux* (Cottidae). The present author was

successful in demonstrating the occurrence of male heterogamety showing an XY-mechanism in the lancelet. It is interesting to see that evidence of a close karyological relationship was obtained again with respect to the sex-chromosome mechanism in the lancelet and same teleosts.

2. Cytological features as seen in the orders of Pisces

The Pisces includes two large groups, Elasmobranchii and Teleostomi. So far as cytological observations have gone, the karyological structure is considerably different between the above two groups. This makes it possible to consider that the Teleostomi may be a group separately divergent in evolution from the Elasmobranchii. In the following, the cytological features will be considered on the basis of available data in several orders of Pisces, with special reference to cytotaxonomical differences.

a. Elasmobranchii

The chromosomes of the Order Laminida have been studied in one species each of the Squalidae and Triakidae. In the Squalidae, Makino (1937) reported on the chromosomes of *Squalus suckleyi* that the diploid complex consists of about 20 atelomitic chromosomes and that the remaining 40 ones which are rod-shaped. In the Triakidae, the present author recorded the chromosome formula in *Mustelus manazo* as 12V's+60r's.

In the Order Rajida, two different karyotypes have so far been reported in the Rajidae and Dasyatidae. The chromosomes of a species of the Rajidae have been investigated by Makino (1937); he gave a chromosome formula of 10r's for *Raja meerdervoortii*. On the contrary, *Dasyatis akajei* belonging to the Dasyatidae has a formula of 20(V's and J's)+64r's.

In conclusion, nothing can be stated generally on the chromosomal relation in Elasmobranchs, since the data are too limited to justify any general statement in relation to evolution.

b. Teleostomi

Based on the karyological data available for consideration, the Order Clupeida is divided into two groups: the Salmonidae and the other group represented by two families, Esocidae and Engraulidae. Although cytological investigations in the Salmonidae have been carried out rather extensively in recent years, the species in which conclusive chromosome data have been secured are few in number. Working with the chromosomes of the Salmonidae, Svärdon (1945) established five different karyotypes in this family: they are (1) 10V's+48r's for *Osmerus eperlanus*, (2) 12V's+48r's for *Salmo salar*, (3) 16V's+64r's for *Salmo trutta*, (4) *Salmo fontinalis* for 16V's+68r's, (5) *Thymallus thymallus* for 28V's+72r's. Bungenberg (1955) gave a formula of 40V's+20r's for *Salmo irideus*, while a formula of 14(V's or J's)+90r's was reported by the present author for the same species. Further, *Oncorhychus keta* is characterized by 20(V's or J's)+54r's (Makino 1937), while *Esox lucius* (Esocidae) and *Engraulis japonicus* (Engraulidae) are represented by a formula, 48r's.

In the Order Cyprinidae, chromosome survey has been made in 25 species of the Cyprinidae, 4 species of the Cobitidae, 1 species of the Siluridae, and 1 species of the Bagridae. There are reported 13 different karyotypes in the Cyprinidae. They are (1) 2V's+42r's for *Rhodeus ocellatus*, (2) 4V's+40r's for *Acheilognathus rhombea*, *A. limbata* and *A. cyanostigma*, (3) 48r's for *Ctenopharyngodon idellus*, *Zacco platypus* and *Hemigrammocypris rasborella*, (4) 2V's+46r's for *Ishikauia steenackeri*, (5) 50r's for *Gnathopogon elongatus*, *Hemibarbus longirostiris*, *Sarcocheilichthys variegatus*, (6) 4V's+46r's for *Acheilognathus lanceolata*, *Pseudorasbora parva*, *Pungtungia herzi* and *Zacco temminckii*, (7) 6V's+44J's for *Abbottina rivularis* and *Tribolodon hakonensis*, (8) 52r's for *Pseudogobio esocinus*, (9) 4V's+48r's for *Barbus semifaciolatus*, (10) 8V's+46r's for *Moroco steindachneri*, (11) 66r's for *Opsariichthys uncirostris*, (12) 94r's for *Carassius auratus* and *C. carassius*, and (13) 104r's for *Cyprinus carpio*. In the Cobitidae, the chromosome formula is given as 4V's+44r's for *Barbatula oreas*, 50r's for *Lefua echigonia*, 52r's for *Misgurnus anguillicaudatus*, and 4V's+50r's for *Cobitis biwae*. *Parasilurus asotus* of the Siluridae has a formula of 58r's, while *Pelteobagrus nudicep* of the Bagridae is characterized by a formula of 56r's.

In the Order Anguillina, the chromosomes of three species have been recorded in the Echelidae and Muraenidae: *Echelus uropterus* shows a formula 4J's+46r's, the formula of *Muraena pardalis* is represented by 14V's+26r's, and *Gymnothorax kidako* possesses a formula of 12V's+30r's.

The Order Cyprinodontida was cytologically analysed rather extensively by Winge (1922), Geiser (1924), Vaupel (1929), Iriki (1932), Katayama (1937), Meyer (1938) and Wickbom (1943). According to them, the chromosomes of 14 species of the Cyprinodontidae are represented by four different karyotypes; *Gambusia halbrooki* and *Moilienisia sphenops* have 36r's, *M. sph.* has 38r's, *Lebistes*, *Heterandria*, *Limia*, *Mollienisia* and *Phallichtys* have a similar formula of 46r's, and *Oryzias* (*Aplocheilus*), *Platypoecilus*, *Rivulus* and *Xiphophorus* are repesented by a formula, 48r's.

In the Order Gasterosteida, *Pungitius tymensis* of Gasterosteidae has been the only representative in which the chromosomes have been investigated, by Makino (1934). He gave a chromosome formula of 42r's for this species.

Considered cytologically, the Order Percida shows a variety of karyological characters. In the Percidae, the chromosome formula is represented by 6V's+4J's+18r's for *Perca fluviatilis*, 8V's+2J's+14r's for *Lucioperca*. In the Anabantidae, *Betta splendens* has a formula of 6V's+36r's. *Coreoperca kawamebari* of the Serranidae and *Sillago shihama* of the Sillanginidae have a formula of 48r's in each. In the Parapercidae the chromosomes of *Cilias pulchella* have been investigated by the present author, giving a formula of 48V's. This type is of quite unique occurrence in the piscian karyotypes in that all the elements are V-shaped. In the Callionymidae, *Callionymus richardsoni* has a formula of 38r's. Three different karyotypes have so far been reported in the Gobiidae: *Tridentiger*, *Gobius similis*, *Acanthogobius*, *Chaenogo-*

bius urotaenia are represented by a chromosome formula of 44r's, while *Gobius abei, Chaenogobius isaza, Boleophthalmus* and *Periophathalmus* have 46r's, and *Mogurnda obscura* has 62r's (XY-type). *Pholis pictus* (pholidae) and *Pseudolabrus japonicus* (Labridae) show a formula of 46r's.

In the Order Tetraodontida, three species of the Aluteridae differ in their chromosome patterns: 34r's for *Stephanolepsis cirrhifer,* 36r's for *Rudarius ercodes* and 40r's for *Novodon modestus* have been reported.

The Order Cottida has been cytologically studied in the following three families, the Synanceiidae, Hexagrammidae and Cottidae: *Inimicus japonicus* (Synanceiidae) shows a formula of 50r's, while *Hexagrammos otakii* (Hexagrammidae) and *Cottus pollux* possess a formula of 48r's in each.

The Order Pleuronectida has been observed cytologically in the Bothidae and Pleuronectidae by the present author. *Paralichthys olivaceus* of the Bothidae has a formula of 46r's, while in the allied family, Pleuronectidae, *Limanda yokohamae* and *Kareius bicoloratus* show a formula consisting of 48r's.

As mentioned in the foregoing pages, the reported evidence indicates that many different karyotypes prevail in the Pisces. But, this survey has made it clear that the most common karyotype which is widely distributed in orders so far cytologically investigated, seems to be a complement in which all the elements are of rod shape, but vary in length. At the present status of cytological investigation, no available data have been presented to show the taxonomical relationship between the related orders. The number of species in which the chromosomes have been studied is very small, and therefore no generalized statement can be made on the evolutional relation of the Pisces. Indeed, the basic data for understanding the evolution of the hereditary relationships among fishes are still incomplete.

3. Cytological features as seen in allied families

In the following, the cytological relationship will be considered in allied families from the cytotaxonomical standpoint. In the Triakidae and Squalidae belonging to the Order Laminida there exists some resemblance or affinity in the cytological character: the chromosome formula of the two families is polymorphic, since the complement contains certain numbers of V- and J-shaped chromosomes. This corresponds to the data derived from the study of morphological characters. The karyotype of the Rajidae, one of the Order Rajida, shows no kinship to that of the closely related family Dasyatidae. Cytological observations yield data which are in parallel to those of the morphological taxonomy.

As regard to the Order Clupeida, the discussion may be based on the Esocidae, Engraulidae and Salmonidae, within the limits to which the cytological observations have gone. Recently, the karyotype of the Esocidae was recorded in *Esox lucius* by Prakken, Bekendam and Pieters (1955); they reported in this species 48 rod-shaped chromosomes with terminal centromeres. The Esocidae and Engraulidae are closely related with each other in their chromosomal features in that they possess identical

karyotypes. This is an item of cytological evidence in favor of their taxonomical kinship. On the other hand, the karyotypes of the Salmonidae are remarkable not only for the high number of chromosomes, but also for the polymorphic nature. At the present status of studies, the family Salmonidae furnishes no cytological evidence which suggests relationship to the above two families. It is most probable that the Salmonidae may be a group separately divergent in evolution from other groups.

In the Order Cyprinida, the Cyprinidae and Cobitidae are remarkable for containing a variety of karyotypes showing close cytological affinity between them, corresponding to the taxonomical relation, though no relationship to other allied families is indicated. The chromosomes of the Siluridae and Bagridae are of telomitic type. The karyotype runs parallel to the morphological and ecological kinship between these two families.

The Echelidae and Muraenidae in the Order Anguillida exhibit a close affinity in their karyological evidence. This feature is fairly parallel to the evidence of morphological taxonomy, since these two families belong to the Suborder Anguillina.

Within the Order Percida, cytological studies have been made on the Percidae and Anabantidae: there is no available data which show cytological relationship between the two families, or to other families. The karyotype of the Callionymidae shows no relationship to that of the other groups, so far as the reported evidence on one species is concerned. The allied families, Serranidae and Sillaginidae, show a close affinity to the Parapercidae and Labridae, in their chromosomal relationship. This suggests a close taxonomical kinship existing between them from the evolutional viewpoint. The Gobiidae shows more close affinity to the Pholidae than to the above four families in chromosomal constitution.

The chromosomes of the Order Tetraodontida have been studied in three species in the Aluteridae. The karyotype of this family consists entirely of telomitic elements, and shows more or less affinity amony the species.

In the Order Cottida, the Hexagrammidae shows more close affinity to the Cottidae than to the Synanceiidae in chromosome constitution, so far as the chromosome pattern is concerned. From this fact it is supposed that they are in close relationship in the taxonomical situation.

The Bothidae and Pleuronectidae in the Order Pleuronectida show a close affinity in karyological relation, corresponding to the data from morphological taxonomy.

As noted the so far in the foregoing descriptions, the cytological data available for the discussion of taxonomical relationship between the related families are at present very limited. Though no generalization is possible in the present status of studies, there are still some items of evidence which show cytological features in favor of the taxonomical relationship.

4. Cytological features between species within a family

In the following families: Engraulidae, Salangidae, Siluridae, Bagridae, Echelidae, Muraenidae, Seranidae, Sillaginidae, Parapercidae, Callionymidae, Labridae, Synanci-

idae, and Bothidae, the chromosome pattern has been investigated in only each one species, and, therefore, no statement can be made on the chromosomal relationship among those families.

Family Salmonidae:— Cytological investigations on the Salmonidae have been carried out rather extensively by several authors such as Prokofieva 1934, Makino 1937, Pomini 1939, Svärdson 1945, Kupka 1948, Bungenberg 1955 and Boothroyd 1957, so that data on the chromosome relation are available for consideration in many species. It should be mentioned, however, that the species in which accurate accounts of chromosomes have been recorded are rather few in number.

Except a slight difference in the chromosome number, the chromosome patterns of *Salmo trutta* and *S. fontinalis* reported by Prokofieva (1934) and Svärdson (1945) are quite in agreement with each other. The accounts of the salmoniid chromosomes given by these two authors are shown in summary in Table 9.

Table 9. Accounts of the chromosomes of some salmoniid fishes reported by Prokofieva (1934) and Svärdson (1945)

	Prokofieva	Svärdson		
	2*n*	2*n*	No. of two-armed chromosomes	*N. F.
Osmerus eperlanus	—	58	10	68
Samo salar	60	60	12	72
Salmo trutta	84	80	16	96
Salmo fontinalis	80	84	16	100
Salmo alpinus	—	80	16	96
Coregonus lavaretus	80	80	16	96
Coregonus albula	—	80	16	96
Thymallus thymallus	—	102	28 ?	130 ?

*N. F.— Total number of chromosome arms (Nobre fondamental).

Svärdson (1945) classified the salmoniid species investigated cytologically by him into three groups, viz., a group with *n*, 29 or 30, a group with *n*, 40 or 42, and a group with *n*, 51. On the above basis, he made the following four-point statement on the distribution of the chromosome number in the Salmonidae:

1), the variation of the chromosome number within the three groups is significant,

2), the difference of the chromosome number between the three groups lies within approximately 10 chromosomes,

3), the chromosome number in each group is almost a multiple of 10, i.e., 30—40—50, and

4), with the increase of the chromosome number, the number of the V-shaped chromosomes increases—contrary to Robertson's law.

Thus, Svärdson (1945) suggested the existence of polyploidy in the salmoniid fishes; the salmoniids represent an old polyploid series, with a basic doubling and not by centric fusion or misdivision. Svärdson's hypothesis has drawn much atten-

tion and repeatedly been discussed by VANDEL (1946), WHITE (1946), KUPKA (1948), MATTHEY (1949), FISHBERG and BEATY (1953), BUNGENBERG (1955) and BOOTHROYD (1957), regarding the occurrence of polyploid relationship in animals.

BUNGENBERG (1955) studied the chromosomes of *Salmo irideus,* and observed 60 chromosomes in cleavage mitosis, of which at least 40 chromosomes have median or submedian centromeres. The proportion between the one-armed and the two-armed chromosomes is highly different from that in the other Salmonidae investigated by SVÄRDSON (1945). Based on the data from *Salmo irideus,* BUNGENBERG suggested that the chromosomes would change in number according to ROBERTSON's law. BOOTHROYD (1957) reported 56 diploid chromosomes in *Salmo salar* from the Canadian populations. This is somewhat lower than the number (*2n,* 60) reported by both PROKOFIEVA and SVÄRDSON for European salmon populations. BOOTHROYD stated that the Canadian populations did not fit directly to SVÄRDSON's hypothesis (1945).

KUPKA (1948) reported 70 or 72 as the diploid number for *Coergonus asperi maraenoides, C. exisguus albellus* and *C. shinzi duplex,* and 36 for *C. wartmanni coeruleus.* He showed that polyploidy occurs in the salmoniid fishes. SVÄRDSON's and KUPKA's arguments in favor of polyploidy in the coregonids are not in agreement with one another, since SVÄRDSON claimed that the species with *2n,* 80 (*Coregonus lavaretus* and *C. albula*) are octoploids with a basic haploid number of 10, while KUPKA regarded the species with *2n,* 70 or 72 as exhibiting tetraploidy by comparison with *C. asperi maraenoides* (*2n,* c. 36), having a basic haploid number of 18. MATTHEY (1949) tends to agree with SVÄRDSON's concept; he stated that even in the case where the evidence of chromosome number appears to be in favor of the hypothesis of polyploidy, the number of major chromosome arms is in disagreement with it.

All of these investigations have dealt with the chromosomes in cleavage mitosis. It is, however, very difficult and at the same time very dangerous to draw any final conclusion as to the chromosomes number from a study of the cleavage material. It is most probable that any work which deals with the chromosome research through all spermatogenetic phases may constitute a valuable contribution to this field. The chromosomes of *Oncorhynchus keta* were previously studied in the primordial germ-cells by MAKINO (1937). He found 76 chromosomes, comprising 20 V-shaped elements and 56 rod-shaped ones. His results are in disagreement with those reported by PROKOFIEVA (1934) and SVÄRDSON (1945) for certain closely related species of the Salmonidae. In the present study, the chromosomes of six salmoniid species were investigated in male germ-cells during spermatogenesis: *Oncorhynchus narka* shows 54 in *n, Salmo irideus* has 104 in *2n* and 52 in *n*; *O. rhodurus, O. masou, O. keta* and *Salvelinus fontinalis* are the same in their haploid number showing 50. Also the results of the present investigations are not in accordance with those reported by the previous authors. In conclusion it may be stated that three genera of this family, *Oncorhynchus, Salmo* and *Salvelinus,* are closely related in their evolutionary origin, so far as the cytological knowledge from the present study is concerned.

Family Cyprindae:— The cytological studies of the Cyprindae up to the present have rather strikingly extended over 26 species covering 19 genera, thanks to the contributions made by Makino (1934, 1941), Matsui, Ojima and Nogusa (1951), and the present author.

The chromosomes of *Rhodeus ocellatus* show more close kinship to those of *Acheilognathus* than to those of the others. This feature corresponds to their taxonomical relationships. *Acheilognathus rhombea, A. limbata* and *A. cyanostigma* are characterized by the same chromosome formula, 4V's+40r's, while *Zacco platypus, Ctenopharyngodon idellus* and *Hemigrammocypris rasborella* are represented by 48r's. If it be assumed that each of the V-shaped chromosomes may be conceived as double, consisting of two rods associated at their inner ends, the total number of chromosomes would be 48 in *Acheilognathus*. Thus it is evident that the formation of multiples by means of centric fusion serves to explain the chromosome relationship between allied species. The same relationship was found to occur between *Ishikauia steenackeri* and *Hemibarbus longirostris* (and *Sarcocheilichthys variegatus* also). If two V's of *Ishikauia* be assumed as light rods, the induced number of chromosomes is 50 in total, which is obtained for *Hemibarbus*. It is then apparent that *Ishikauia* and *Hemibarbus* are taxonomically closely related to each other.

Certainly the karyological similarity here demonstrated suggests a close genetic relationship between them and indicates at the same time their taxonomical affinity. *Gnathopogon elongatus elongatus* and *G. elongatus caerulescens* are clasely related with each other in respect to their chromosomes by possessing identical karyotypes, 50r's, so far as the number and other morphological features of the chromosomes are concerned. A similar situation seems to occur between *Moroco steindachneri* and *M. percnurus*. However, the formula is 48r's for *Zacco platypus*, and 4V's+46r's for *Zacco temmincki*; those two species are dissimilar in their chromosome constitution, in spite of the fact that they are in a close relationship in the taxonomical situation. There exists a close relationship in respect to the chromosomes between *Zacco temmincki, Pseudorasbora parva parva, Acheilognathus lanceolata* and *Pungtungia herzi*. It seems most probable that cytological sequence may be a genetical expression of their taxonomic kinship.

Pseudogobio esocinus and *Opsariichthys uncirostris* are characterized by possessing a karyotype composed of rod-shaped elements of telomitic nature, notwithstanding that the number of chromosomes differ from one another, being 52 ($2n$) in *Pseudogobio* and 66 in *Opsariichthys*. *Barbus semifaciolatus* is represented by a chromosome formula, 4V's+48r's. The karyotype of this form is unique and furnishes a new example for the Cyprinidae.

A peculiar type has been found in two species of this family, *Tribolodon hakuensis* (Makino 1939) and *Abbottina rivularis* (Nogusa 1955). According to Makino (1939), the karyotype *Tribolodon hakuensis* is characterized by showing three pairs of V-shaped elements and the remaining 44 J-shaped ones of subterminal structure.

The comparison of *Abbottina rivularis* with *Tribolodon* reveals that there is a close similarity between the two species, in both the number of chromosomes and the structure of the karyotype.

A close kinship between the Funa (*Carassius carassius*) and the gold-fish (*C. auratus*) was proved on both the serological and genetical basis (ISHIHARA and MISAO 1929, MATSUI 1934), and verified again from karyological investigations by the finding that a very similar chromosome pattern exists through the Funa and the gold-fish varieties (MAKINO 1941). They easily cross and yield offspring with normal fertility (MATSUI 1934). Closely allied to the Funa is the carp (*Cyprinus carpio*). When kept under confined conditions, the carp and the Funa (and the gold-fish also) mate and can produce hybrids (MAKINO, OJIMA and MATSUI 1955). Reciprocal crosses are also possible with similar results. The carp and the Funa are strikingly dissimilar in their chromosome patterns, not only in the number but also in the morphology of the chromosomes (MAKINO 1939, 1941). In diploid the carp show 104 chromosomes, which are all thick, short, rod-shaped elements with no marked size differences, while in the Funa, and in the gold-fish as well, there are 94 diploid chromosomes which are characterized by a slender rod-shape showing a slight variation in length. The chromosomes of the hybrids as observed in spermatogonial division are 99 in number, being the total sum of the haploid numbers of the parents, 52 for the carp and 47 for the Funa (MAKINO, OJIMA and MATSUI 1955).

Family Cobitidae:— The chromosomes of the Cobitidae have been known in four forms. The karyotypes of *Misgurnus anguillicaudatu* and *Barbatula oreas* have been studied in detail by MAKINO (1941). According to him, the chromosome number of *Misgurnus* is 52, all the chromosomes being rod-shape with terminal centromere, while in *Barbatula* it is reduced to 48, but they include two pairs of V-shaped chromosomes. On the basis of ROBERTSON's law (1916), the numerical difference between the above two species may be explicable as the result of the formation of multiple through centric fusion of two rods. The chromosome formula was represented as 50r's for *Lefua echigonia,* and as 4V's+50r's for *Cobitis biwae.* Evidence indicates that the karyotype of *Lefua* shows a slight affinity to that of *Misgurnus* rather than to that of *Cobitis.* Further it is noticeable that the karyotype of *Cobitis* is highly different from those of any other species of this family.

The Cobitidae show a close affinity to the Cyprinidae in their chromosomal relationship. Thus, it can be said that the Cyprinidae and Cobitidae are closely related, in agreement with the opinion of MATSUBARA (1955) who emphasized that the Cobitidae are regarded as belonging to a subgroup of the Cyprinidae.

Family Muraenidae:— The chromosomes of two species in this family have been investigated by the present author. The chromosome formula was 14V's+26r's for *Muraena pardalis,* and 12V's+30r's for *Gymnothorax kidako.* The numerical relation between the above two forms is such that it can be explained by the mechanism of centric fusion. It is evident that there is a close relationship between

Muraena and *Gymnothorax*. The chromosomes of the Muraenidae are highly different from those of the allied families by having a low number. It is probable that this family may be separately divergent in evolution from other families.

Family Gobiidae:— In the Gobiidae, the chromosomes of nine species have been reported by the present author (1950, 1955, 1957). *Tridentiger obscurus, Gobius similis, Acanthogobius flavimanus* and *Chaenogobius urotaenia* are characterized by the same chromosome formula, 44r's, while *Gobius abei, Chaenogobius isaza, Boleophthalmus pectinirostris* and *Periophthalmus cantonensis* are represented by 46r's. The diploid number for the formers forms is 44, two less than those for the latter forms. The diploid complements of the above species contain small sized chromosomes referred to as m-chromosomes, with the exception of *Gobius similis*. Each species is characterized by possessing a definite number of such m-chromosomes. The reduction of the chromosome number which occurs between *Gobius similis* and *Gobius abei*, and between *Chaenogobius urotaensa* and *C. isaza* seems to be due to the disappearance of the m-chromosome. Considered from this fact, it seems probable that the gradual reduction of size may occur in the m-chromosomes from species to species with final disappearance in the extreme case. In summary, these species are closely related with each other in cytological and genetical constitutions, irrespective of the fact that they differ in their morphological characters.

An exception occurs in *Mogrunda obscura*. The male chromosome formula of this species is 62r's. Remarkable is the occurence of the sex-chromosomes of the XY-mechanism in this species. The karyotype of *Mogrunda* shows no relationship to those of the others of the family at all. This corresponds to the data derived from the study of morphological characters, because of the fact that *Mogrunda obscura* shows a closer kinship to the Eleotridae that to the other species of the Gobiidae.

Family Aluteridae (Monacanthidae):— The chromosomes of the Aluteridae have been known in the following three forms, *Stephanolepis cirrhifea, Rudarius ercodes* and *Novodon modestus*. *Stephanolepis cirrhifer* has a chromosome formula of 34r's, and *Rudarius ercodes* possesses 36r's, while *Novodon modestus* shows the formula with 40r's. In comparison of the chromosomes between the above three forms, it can be stated that *Stephanolepis cirrhifer* shows more affinity to *Rudarius ercodes* than to *Novodon modestus*. This is in parallel with the morphological taxonomy.

Family Hexagrammidae:— The chromosomes of two related forms, *Hexagrammos octogrammus* (Makino 1937) and *H. otakii* show 48 in $2n$, and 24 in n. All the chromosomes are characterized by a simple rod-type telomitic nature. These two species show a fair agreement in that the haploid number is 24, being parallel to their taxonomical kinship.

Family Cottidae:— In this family, the chromosomes of the two species, *Cottus bairdii* and *Cottus pollux*, have been investigated. *Cottus bairdii* possesses $2n$, 36—

38, *n*, 18 and *Cottus pollux* has 2*n*, 48, *n*, 24. There is a striking difference in the chromosome number between the two, in disregard of their taxonomical affinity.

Family Pleuronectidae:— The chromosomes of two species, *Limanda yokohamae* and *Kareius bicoloratus*, of this family have cytologically been investigated by the present author. Both species are entirely alike in their chromosome formula by showing 48r's. This cytological evidence seems to support the taxonomical kinship occurring between them.

5. Cytological evidence of the sex-determining mechanism in fishes

The mechanism of sex-determination in fishes has been discussed and emphasized from the genetical standpoint by several geneticists, particularly on the basis of the sex-linked inheritance studied in the Cyprinodontidae. AIDA (1921) in the Japanese killifish, *Oryzias* (*Aplocheilus*) *latipes*, WINGE (1922, 1923) in *Lebistes reticulatus*, and CHAVIN and GORDON (1951) in *Platypoecilus maculatus*, obtained genetic results which indicate the occurrence of male heterogamety, while BELLAMY (1923, 1928), GORDON (1927),and FRASER and GORDON (1929) furnished evidence that shows a homogametic condition of the male in poecilied fishes. Efforts have been made by many cytologists to obtain a cytological demonstration for above genetic data during some twenty years (WINGE 1922, 1923, VAUPEL 1929, RALSTON 1934, FOLEY 1926, BENNINGTON 1936, MAKINO 1934, 1937, 1939, 1941 and WICKBOM 1943).

On the basis of his genetic experiments, WINGE (1922) has suggested the possibility of the presence of heteromorphic chromosomes forming an XY-mechanism in the male of *Lebistes*. Working on *Umbra*, FOLEY (1926) described a pair of large L-shaped chromosomes in the spermatogonium which he regarded an XY-pair of sex-chromosomes. The same view has been emphasized by BENNINGTON (1936) who found a pair of large chromosomes in the spermatogonium of *Betta splendes*. In the study of *Lebistes*, VAUPEL (1929) has described a pair of large chromosomes which migrate to the pole precociously in the first division, and stated that there is some evidence for the presence of an XY-pair. RALSTON (1934), working with *Platypoecilus*, *Xiphophorus* and their hybrids, has found a pair of large chromosomes which migrate to the poles of the spindle in the first division; he indicated that those chromosomes might be the sex-chromosomes of an XX-type in the male.

As one looks over these reported cases, he immediately accepts the idea that the chromosomal elements indicated by those previous authors as the sex-chromosomes are no other than abnormally protruding ordinary bivalents caused by faulty preservation. Very often, one can encounter not certainly identifiable chromosomes which got abnormally protruded from the equatorial plate in poorly fixed material. Neither in behavior nor in structure, they are the so-called sex-chromosomes comparable to those generally found in insects and mammals. Prior to any conclusive statement, one should notice that such aberrations as above are likely to be caused by inadequate fixation, as repeatedly claimed by MAKINO (1934, 1939, 1941) in his papers on the chromosomes of some teleosts. The evidence presented by those previ-

ous authors is, therefore, never conclusive for the demonstration of the sex-chromosomes.

On the contrary to the above cases, some authors have failed to find out any particular chromosomes. No unpaired elements occur in the diploid complement: at meiosis, there is no indication of heteropycnosis in the growing nucleus, no lagging or advancing chromosome, or any other feature suggesting the existence of the sex-chromosome during the course of cell division. Such a conclusion was reached through the investigations of Iriki (1932), Makino (1934, 1937, 1939, 1941), Wickbom (1943), Svärdson and Wickbom (1939) and Nogusa (1943, 1950, 1951a, 1951b, 1955, 1957) in various forms of teleost fishes.

In striking contrast to the above cases so far recorded, the present author was successful for the first time in demonstrating clear-cut and definite evidence for the occurrence of the sex-chromosomes in fishes: in 1955 the author found a clear-cut XY-mechanism to occur in the male of a species of the Gobiidae, *Mogrunda obscura* (Nogusa 1955). In addition, a cytological demonstration of male heterogamety was also successful by the present author (Nogusa 1956) in *Cottus pollux* (Cottidae). As mentioned in the foregoing descriptions, the X-chromosome is represented by one of the large chromosomes, and Y by one of the small chromosomes in the above two species. Though there are some difficulties in identifying the sex-elements in both the spermatogonia and secondary spermatocytes, the following facts are strong evidence for the existence of the XY-chromosomes in male germ cells: that is, 1) the XY-complex can be completely traced as definite heteropycnosis bodies throughout the growth period, 2) the XY-bivalent is clearly distinguishable from the autosomal bivalents on account of its characteristic, 3) the XY-complex is extremely characteristic in the mode of conjugation, and 4) the X segregates from the Y in the first division and they migrate to opposite poles. It is thus apparent that the cytological evidence here presented is quite conclusive in respect to the claim on the occurrence of male heterogamety in *Mogrunda obscura* and *Cottus pollux*.

Here, it should be considered why the sexual difference of chromosomes has not been demonstrated in many other forms of fishes studied by several authors, as cited above. Most probably, the sex-chromosomes of many fishes are not generally so much differentiated that they acquire characteristic behavior, or are distinguishable from other autosomes, and accordingly the sex-chromosomes cannot be detected with morphologically differentiated features.

Recently, cytological male heterogamety has been reported by Yosida (1956) to occur in an anuran amphibian, *Hyla arborea japonica*. Using his improved squash technique, he demonstrated the sex-chromosomes of an XY-type. There are evidences indicating that the sex-chromosomes of lower vertebrates are not always morphologically indistinguishable from the autosomes. The findings of the present study and of Yosida (1956) offer clear evidence favorable to the above view. Niiyama (1937, 1938, 1941) has established male heterogamety in certain species of decapod crustace-

ans, such as *Plagusia dentipes, Eriocheir japonicus, Hemigrapsus sanguineus* and *Ovalipes puncatus,* by finding a clear-cut XY-complex or an X-element, while in many species he has failed to demonstrate the sex-chromosomes. Thus the decapod crustaceans are interesting in furnishing a situation very comparable to that occurring in fishes.

As noted above, the genetic evidence on the mechanism of sex-determination has been left still in confusion with regard to the cyprinodont fishes. Some authors have obtained results which show a heterogametic condition in the male (AIDA 1921, WINGE 1922, 1923, CHAVIN and GORDON 1951), while some others have claimed the occurrence of female heterogamety in poeciliid fishes (BELLAMY 1923, GORDON 1927, FRASER and GORDON 1929). Whatever the genetic evidence may be, the findings of the present investigation are of interest and importance in furnishing clear-cut cytological evidence for male heterogamety in gobiid and in cottiid fishes by demonstrating a XY-mechanism.

6. Variation of the chromosome number due to a multiple chromosome formation

Definite variation of the chromosome number which is explicable as a result of the formation of multiple chromosomes by centric fusion, probably through the process of translocation (or translocations), seems to occur rather frequently among certain related forms of animals. The best example of such a chromosome-linkage has been demonstrated in acridian grasshoppers by MCCLUNG (1905, 1917) and ROBERTSON (1916). It is generally accepted at present that such a mechanism has probably played a significant part in the permanent change of chromosome number from species to species.

According to MCCLUNG (1930), there are two general types of multiple formation: in one, the variation of the chromosome number takes place as a result of the union of chromosomes among individuals within the same species; in the other, the variation is fixed as a practically permanent feature within the taxonomic group. Many examples so far reported belong to the latter type. While working with piscian chromosomes, the present author found a clear-cut example of the former type in a cyprinid fish (*Acheilognathus rhombea*), which seems to be the first and only account of such a chromosome variation in vertebrates to date.

The fishes (*Acheilognathus rhombea*) used as material for the present investigation were collected in the same locality. As described above in detail, three karyotypes, viz., 4V-type, 2V-type and NO V-type, were found to occur in this material. Of remarkable and particular interest is the fact that a single individual is provided with two or three karyotypes, and further that the distribution of different karyotypes varies by individuals (Table 3—5). The variation in karyotypes, as well as the numerical variation in chromosomes, can be accounted for on the basis of the formation of V-shaped multiple chromosomes by centric union, probably due to a process of translocation or translocations.

No positive data can be presented here concerning the mechanism of the formation of multiple chromosomes. As generally considered, the mechanism may be due to mutual translocation, taking place at the centromere regions of certain chromosomes (White 1954). If this consideration be accepted as probable the prototype of this species may possess a chromosome complex of the NO V-type.

Chromosamal relationships of a similar type are not rare between related forms of vertebrates (Makino 1951); examples have been reported between allied species of urodelan amphibians (Makino 1932, 1935), lizards (Matthey 1939, Makino and Asana 1948, Makino and Momma 1949), birds (Yamashina 1943, 1945, Yamashina and Makino 1946, Udagawa 1954) and mammals (Makino 1943). In teleosts, a quite analogous case was found by Makino (1941) between two allied forms of the Cobitidae: *Misgurunus anguillicaudatus* shows a diploid number of 52, consisting of all telomitic elements, while in *Barbatula oreas* it is reduced to 48, consisting of four V-shaped elements while the remainder are rod-shaped ones. The chromosomal variation between the two species is such as to be explicable by the formation of V-shaped multiples.

A similar relationship of the chromosomes has been found in some other cyprinids. The chromosome number of *Acheilognathus cynanostigma* is 44, consisting of 4V's and 40 rods (Nogusa 1951a). Apparently the karyotype of this species is the same as the 4V-type of *Acheilognathus rhombea*. The chromosomes of the following three species: *Zacco platypus* (Nogusa 1951b), *Hemigrammocypris rasborella* (Nogusa 1954) and *Ctenopharyngodon idellus* (Matsui, Ojima and Nogusa 1951), uniformly possess 48 chromosomes in diploid, all being of a simple rod-type. The chromosome complex of these three forms is nearly identical with that of the NO V-types of *A. rhombea*, both in number and morphology. Further, a quite analogous case was found by the present author between two related species of the Muraenidae, *Muraena pardalis* and *Gymnothorax kidako:* the chromosome complex of the former (2*n*, 40) is provided with seven pairs of V's, thirteen pairs of rods of intergrading sizes (14V's+26r's), while in *G. kidako* (2*n*, 42), it is composed of six pairs of V's fifteen pairs of short rods (12V's+30r's). Closer investigation shows that the chromosomal variation between these related species is fairly explicable by the mechanism of centric fusion. Thus the idea of the permanent formation of multiples by centric fusion serves to explain the chromosome relationship between allied species.

7. Occurrence of univalent chromosomes

Occasional occurrence of univalent chromosomes at first metaphase, which is probably due to the failure of meiotic chromosome pairing, has been clearly as certained among plants, especially in plant hybrids. In animals, Makino (1941) has observed the occurrence of univalent chromosomes in nine varieties of gold-fish (*Carassius auratus*). He has stated that the gold-fish varieties are of hybrid origin having been produced by varietal crosses and that some have arisen as a result of selection during many years by breeders, and that the univalents found in gold-fish

varieties may be produced on the basis of the failure of meiotic pairing of chromosomes.

Recently, WHITE (1949, 1951a, 1951c), EVANS (1954) and OHMACHI and UESHIMA (1957) reported in certain orthopteran insects the variation of chromosome number due to the occurrence of univalents in wild populations or in individuals within the same species from different localities. OHMACHI and UESHIMA (1957) observed chromosomes in germ-cells of some crickets which precociously divide and go to one pole or the other.

The present author also found a corresponding feature in *Inimicus japonicus* (Synanceiidae), showing that cells with unpaired chromosomes occur in a high frequency at first meiosis. Numerical variation due to the inclusion of univalents in primary and secondary spermatocytes was found to occur within a single individual from the same locality. With regard to the cause of the precocious disjoining of the univalent chromosomes in the first meiotic division, WHITE (1941) and HUGHES-SCHRADER (1943, 1950) have attributed it to the premetaphase stretch which gives rise to repulsion between a pair of homologous chromosomes at an early stage of the first meiosis. A similar view was expressed by SMITH (1953) in his observations on some Indian crickets. The present author has hitherto failed to observe premetaphase stretch in *Inimicus*. Thus, nothing can be stated at present on the cause of the formation of univalents.

Summary

The present paper describes the results of a comparative study of the chromosomes in 64 species of the primitive and lower chordates, such as one species of the lancelet, three species of cyclostomes and 60 species of fishes, with special regard to the taxonomical and evolutional significance of cytological chracters.

The descriptions of the data were presented according to the arrangement of current taxonomy; 64 species cytologically investigated in the present study represent 53 genera which cover 26 families and 12 orders. The numerical and morphological features of the chromosomes which were established for each species are tabulated in Table 10.

On the basis of the karyological evidence, the taxonomical and evolutional relation of the lancelet to lower vertebrates was considered. From the cytological basis here established, it seems probable that there does exist a certain phylogenetic relationship between the lancelet and lower vertebrates.

The occurrence of a sex-chromosome of an XY-mechanism was demonstrated in the Japanese lancelet, *Branchiostoma belcheri*. A sex-chromosome of the same type was established to occur in two forms of teleost fishes, one in *Mogrunda obscura* (Gobiidae) and the other in *Cottus pollux* (Cottidae). Thus, evidence of a close karyological relationship between the lancelet and lower vertebrates was obtained again with respect to the sex-chromosome mechanism.

Viewed generally, the mechanism of sex-determination in the vast majority of teleosts is still in an undifferentiated primitive state; the sex-chromosomes are very little differentiated from the autosomes, and therefore they are not distinguishable morphologically from the latter.

Generally speaking, there are considerable variations in shape and size of the chromosomes among the fishes so far studied. The general tendency is that there occurs a similarity in number and shape of chromosomes between or among taxonomically related forms of fishes. The similarity and dissimilarity of the chromosome patterns serve as useful criteria for the distinction of one species from its relatives, or for the consideration of the phylogenetic relationship of species.

It has been shown that the cytological mechanism called centric fusion, which is explicable as a result of association of two non-homologous rod-shaped chromosomes to form a V-shaped multiple probably through the process of translocation or translocations, plays also a significant role in the course of the evolution of fishes. Further, there has been presented some data showing that the reduction of the chromosome number is caused by the lack of the smallest chromosomes, so-called m-chromosomes; the gradual reduction in size of m-chromosomes seems to constitute one of the possible modes of numerical variation in fishes.

Table 10. The species dealt with in this study and their chromosome numbers and formulae established by the present author

Species name	Chrom. Number		Fomula
	$2n$	n	
Cephalochordata			
A. Branchiostomidae			
1. *Branchiostoma belcheri*	32s	16(I, II)	32r
Cyclostomi			
B. Eptatretidae			
2. *Eptatretus burgeri*	48s	24(I, II)	48r
3. *Eptatretus okinoseanus*	46s	23(I, II)	46r
C. Petromyzonidae			
4. *Entosphenus reissneri*	94—96s		94r—96r
Elasmobranchii			
D. Trikidae			
5. *Mustelus manazo*	72s	36(I)	12(V & J)+60r
E. Dasyatidae			
6. *Dasyatis akajei*	84s	42(I)	20(V & J)+64r
Teleostomi			
F. Engraulidae			
7. *Engraulis japonicus*	48s	24(I)	48r
G. Salmonidae			
8. *Oncorhynchus nerka*		54(I)	
9. *Oncorhynchus rhodurus*		50(I)	
10. *Oncorhynchus masou*		50(I)	
11. *Oncorhynchus keta*		50(I)	
12. *Salmo irideus*	104s	52(I)	14(V & J)+90r
13. *Salvelinus fontinalis*		50(I)	

H. Salangidae			
14. *Salangichthys microdon*		28 (I)	
I. Cyprinidae			
15. *Rhodeus ocellatus*	44s	22 (I, II)	2V + 42r
	44s	22 (I, II)	4V + 40r
16. *Acheilognathus rhombea*	46s	23 (I, II)	2V + 44r
	48s	24 (I, II)	48r
17. *Acheilognathus limbata*	44s	22 (I, II)	4V + 40r
18. *Acheilogdathus cyanostigma*	44s	22 (I, II)	4V + 40r
19. *Pungtungia herzi*	50s		4V + 46r
20. *Sarcochielichthys variegatus*	50s	25 (I, II)	50r
21. *Abbottina rivularis*	50s	25 (I, II)	6V + 44J
22. *Gnathopogon elongatus elongatus*	50s	25 (I)	50r
23. *Gnathosogon elongatus caerulescens*	50s	25 (I)	50r
24. *Pseudogobio esocinus*	52s	26 (I, II)	52r
25. *Hemibarbus longirostris*	50s	25 (I, II)	50r
26. *Ctenopharyngodon idellus*	48s	24 (I)	48r
27. *Moroco steindachneri*	54s	27 (I)	8V + 46r
28. *Moroco percnurus*		27 (I, II)	
29. *Zacco platypus*	48s	24 (I, II)	48r
30. *Zacco temmincki*	50s		4V + 46r
31. *Opsariichthys uncirostris*	66s	33 (I, II)	66r
32. *Hemigrammocypris rasborella*	48s	24 (I, II)	48r
33. *Ishikauia steenackeri*	48s	24 (I)	2V + 46r
34. *Barbus semifaciolatus*	52s	26 (I)	4V + 48r
J. Cobitidae			
35. *Lefua echigonia*	50s	25 (I)	50r
36. *Cobitis biwae*	54s	27 (I)	4V + 50r
K. Siluridae			
37. *Parasilurus asotus*	58s	29 (I)	58r
L. Bagridae			
38. *Pelteobagrus nudiceps*	56s	28 (I)	56r
M. Echelidae			
39. *Echelus uropterus*	50s	25 (I)	4J + 46r
N. Muraenidae			
40. *Muraena pardalis*	40s	20 (I)	14V + 26r
41. *Gymnothorax kidako*	42s	21 (I)	12V + 30r
O. Serranidae			
42. *Coreoperca kawamebari*	48s	24 (I)	48r
P. Sillaginidae			
43. *Sillago sihama*	48s	24 (I, II)	48r
Q. Parapercidae			
44. *Cilias pulchella*	48s	24 (I)	48V
R. Callionymidae			
45. *Callionymus richardsoni*	38s	19 (I)	38r
S. Gobiidae			
46. *Mogrunda obscura*	62s	31 (I, II)	62r
47. *Tridentiger obscurus*	44s	22 (I, II)	44r
48. *Gobius similis*	44s	22 (I)	44r
49. *Gobius abei*	46s	23 (I)	46r
50. *Acanthogobius flavimanus*	44s	22 (I, II)	44r
51. *Chaenogobius urotaenia*	44s	22 (I)	44r
52. *Chaenogobius isaza*	46s	23 (I)	46r
53. *Boleophthalmus pectinirostris*	46s	23 (I)	46r
54. *Periophthalmus cantonensis*	46s	23 (I)	46r

T. Labridae			
55. *Pseudolabrus japonicus*	46s		2V+44r
U. Aluteridae (Monacanthidae)			
56. *Stephanolepis cirrhifer*	34s	17(I)	34r
57. *Rudarius ercodes*	36s	18(I)	36r
58. *Novodon modestus*	40s	20(I)	40r
V. Synanceiidae			
59. *Inimicus japonicus*	50s	25(I, II)	50r
W. Hexagrammidae			
60. *Hexagrammos otakii*	48s	24(I)	48r
X. Cottidae			
61. *Cottus pollux*	48s	24(I, II)	48r
Y. Bothidae			
62. *Paralichthys olivaceus*	46s	23(I)	46r
Z. Pleuronectidae			
63. *Limanda yokohamae*	48s	24(I)	48r
65. *Kareius bicoloratus*	48s	24(I)	48r

s : spermatogonium. I: first spermetocyte. II: secondary spermetocyte.
V: V-shaped chromosome. r: rod-shaped chromosome.
J : J-shaped chromosome.

Literature Cited

Aida, T. 1921. On the inheritance of colour in a fresh-water fish, *Aplocheilus latipes*, with special reference to sex-linked inheritance. *Genetics* **6**: 554–573.

———. 1930. Further genetical studies of *Aplocheilus latipes*. *Genetics* **15**: 1–16.

———. 1936. Sex-reversal in *Apliocheilus latipes* and a new explanation of sex-differentiation. *Genetics* **21**: 136–153.

Bennington, N. L. 1936. Germ cell origin and spermatogenesis in the Siamese fighting fish, *Betta splendens*. *Jour. Morph.* **60** : 103–125.

Boothroyd, E. R. 1957. The chromosomes of spring- and fall-run Atlentic Salmon. *Proceed. Genet. Society. Canada.* **2**: 41.

Bungenberg de Jong, C. M. 1955. Cytological studies in *Salmo irideus*. *Genetica* **27**: 472–485.

Chavin, W. and Gordon, M. 1951. Sex-determination in *Platypoecilus maculatus*. Differentiation of the gonads in members of all male broods. *Zoologica* **36**: 135–146.

Evans, W. L. 1954. Cytology of the grasshopper genus *Circotettix*. *Amer. Nat.* **88**: 21–32.

Fischberg, M. and Beatty. R. A. 1952. Heteroploidy in mouse embryo's due to crossing of inbred strains. *Evolution* **6**: 316–325.

Foley, J. O. 1926. The spermatogenesis of *Umbra limi* with special reference to the behaviour of the spermatogonial chromosomes and the first maturation division. *Biol. Bull.* **50**: 117–147.

Friedman, B. and M. Gordon. 1934. Chromosome number in Xiphophorin fishes. *Amer. Nat.* **68**: 446–455.

Geiser, S. W. 1924. Sex ratio and spermatogenesis in the topminnow, *Gambusia holbrooki* Grd. *Biol. Bull.* **47**: 175–212.

Gregory, W. K. 1951. Evolution Emerging. New York.

Hann, H. 1927. The history of the germ cells of *Cottus bairdii* Grd. *Jour. Morph.* **43**: 427–498.

Hughes-Schrader, S. 1943. Polarization, Kinetochore movements, and bivalents, and bivalents structure in meiosis of male mantids. *Biol. Bull.* **85**: 265–300.

———. 1950. The chromosomes of mantids (Orthoptera: Mantidae) in relation to taxonomy. *Chromosoma* **4**: 1–55.

Iriki, Sh. 1932. Preliminary notes on the chromosomes of Pisces. I. *Aplocheilus latipes* and *Lebistes reticulatus*. *Proc. Imp. Acad.* (Tokyo). **8**: 262–263.

———. 1932. On the chromosomes of *Aplocheilus latipes*. *Sci. Rep. Tokyo Bunrika Daigaku, Sec. B.* **1**: 127–131.

Ishihara, M. and T. Misao. 1929. Serological studies on hybrids between carp, Crucian carp, and gold-fish (in Japanese). *Jap. Jour. Genet.* **4**: 147–150.

Kastschenko, W. 1890. Über den Reifungsprozetz des Selachiereies. *Zeits. wiss. Zool.* **50**: 428.

Kichjio, H. 1939. Chromosomes of *Tachopteryx peryeri* and *Gomphus hakinsis* (Odonta: Aeschnidae). *Jap. Jour. Genet.* **15**: 287–289.

Kupka, E. 1948. Chromosomal Vershiedenheiten bei Schweizerishen Coregenen. *Rev. Suiss Zool.* **55**: 285–293.

Makino, S. 1932. The chromosomes number in some salamanders from northern Japan. *Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool.* **2**: 97–108.

———, 1934. The chromosomes of the sticklebaks, *Pungitius tymensis* and *P. pungitius*. *Cytologia* **5**: 155–168.

———. 1935. The chromosomes of *Cryptobranchus allegheniensis*. *Jour. Morph.* **58**: 573–583.

———. 1937. Notes on the chromosomes of some teleost fishes. *Zool. Mag.* (Japan). **49**: 75–76.

———. 1939. The chromosomes of the carp, *Cyprinus carpio*, including those of some related species of Cyprinidae for comparision. *Cytologia* **9**: 430–440.

———. 1941. A karyological study of gold-sfih of Japan. *Cytologia* **12**: 96–111.

———. 1941. The chromosomal relation between the two allied species of the loach (Cobitidae, Pisces). *Cytologia* **12**: 79–82.

———. 1941. Studies on the murine chromosomes, I. Cytological investigations of mice included in the genus *Mus*. *Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool.* **7**: 305–380.

———. 1942. Studies on the murine chromosomes, II. Morphological comparison of the chromosomes between the wild form and the domesticated variety of *Rattus norvegicus* (Berkenhout). *Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool.* **8**: 31–43.

———. 1943. Studies on the murine chromosomes, III. A comparative study of chromosomes in five species of Rattus. *Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool.* **9**.

———. 1944. Karyotypes of domestic cattle, zebu and domestic waterbuffalo (Chromosome studies in domestic Mammal, IV). *Cytologia* **12**: 247–264.

———. 1948. Secondary association of chromosomes in *Palpares pardus* var. *asanai* Kuwayama. *Oguma Comm. Vol. Cytology and Genetics*. 59–62.

———. 1956. A review of the chromosome numbers in animals. Tokyo.

Makino, S. and Asana, J. J. 1948. A sexual difference in the chromosomes of two species of agamid lizards. *Chromosoma* **3**: 208–219.

Makino, S. and Momma, E. 1949. An idiogram study of the chromosomes in some species of reptiles. *Cytologia* **15**: 96–108.

Makino, S., Y. Ojima and Y. Matsui. 1955. Some cytological features of sterility in the Carp–Funa hybrids. *Annot. Zool. Japon.* **28**: 12–16.

Matsui, Y. 1934. Genetical studies on gold-fish of Japan. I. On the varieties of gold-fish and the variations in their external characteristic. *Jour. Imp. Fish. Inst.* **30**: 1–36.

Matsui, Y., Y. Ojima and S. Nogusa. 1952. Chromosomes of *Ctenopharyngodon idellus* (Cyprinidae, Pisces). *Zool. Mag.* **61**: 288–289.

Matsubara, K. 1955. Fish morphology and Hierarchy. Tokyo.

Matthey, R. 1939. La loi de Robertson et la formule chromosomiale chez deux Lacertiens: *Lacerta ocellata* Daud., *Psammodromus hispanicus* Fitz. *Cytologia* **10**: 32–39.

———. 1947. Quelques formules chromosomiales. *Scientia Genetica* **3**.

———. 1949. Les Chromosomes des Vertébrès. Lausanne: Rouge.

McClung, C. E. 1905. The chromosome complex of orthopteran spermatocytes. *Biol. Bull.* **9**: 304–340.

———. 1908. Cytology and Taxonomy. *Kansas Univ. Bull.* **4**, No. 7.

———. 1917. The multiple chromosomes of *Hesperotettix* and *Mermiria* (Orthopera). *Jour.*

Morph. **29**: 519-605.

———. 1930. Multiple chromosome in the Orthoptera. *Act. Cong. Intern. Biol. Montevideo, fasc.* **VII**: 1841-1848.

Minouchi, O. 1936. Natiz über die Chromosomen von *Tethyum plicatum* Les (Ascidia). *Z. Zellforsch. mikr. Anat.* **23**: 790-794.

Momma, E. 1943. A karyogarm study on 18 species of Japanese Acrididae (Orthoptera). *Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool.* **9**: 59-69.

Moore, J. E. S. 1895. On the structural changes in the reproductive cells during the spermatogenesis of elasmobranchs. *Quart. Jour. Micr. Sci.* **38**: 275.

Niiyama, H. 1937. The problem of male heterogamety in the decapod Crustasea, with special reference to the sex-chromosomes in *Plagusia dentipes* de Haan and *Eriocheir japonicus* de Haan. *Jour. Fac. Sci, Hokkaido Imp. Univ. Ser. VI, Ser. VI, Zool.* **5**: 283-295.

———. 1938. The XY-chromosomes of the shore-crab, *Hemigrapsus sanguineus* (de Haan). *Jap. Jour. Genet.* **14**: 33-38.

———. 1941. The X-O type of sex-chromosomes found in *Ovalipes punctatus* (de Haan) (Crustacea : Decapoda). *Jap. Jour. Genet.* **17**: 41-45.

Nogusa, S. 1943. On the chromosomes of four species of the Cyprinidae (Pisces). (in Japanese with English resume). *Jap. Jour. Genet.* **19**: 282-287.

———. 1950. Chromosome Studies in Pisces, I. On the chromosomes of two species of the Gobiidae. (in Japanese with English resume). *Jap. Jour. Genet.* **25**: 153-157.

———. 1951. Chromosome Studies in pisces, II. On the chromosomes of *Sillago sihama* (Sillaginidae) and *Parasilurus asotus* (Siluridae). (in Japanese with English resume). *Jap. Jour. Genet.* **26**: 153-155.

———. 1951. Chromosome Studies in Pisces, III. The chromosomes of four species of the Cyprinidae. *La Kromosomo* **11**: 421-424.

——— . 1953. List of the chromosome complements in the Cyprinidae. *Science Rep. Hyogo Univ. Agricul. Series Natural Science* **1**: 1.

———. 1953. A study of the chromosomes of some lizards, with special remarks on the m-chromosomes. *Cytologia* **18**: 29-35.

———, 1954. Notes on the chromosomes of *Abbottina rivularis* (Cyprinidae). *Jap. Jour. Genet.* **30**: 10-11.

———. 1955. Chromosome Studies in Pisces, IV. The chromosomes of *Mogrunda obscura* (Gobiidae), with evidence of male heterogamety. *Cytologia* **20**: 11-18.

———. 1955. Chromosome Studies in Pisces, V. Variation of the chromosome number in *Acheilognathus rhombea* due to multiple-chromosome formation. *Annot. Zool. Japon.* **28**: 249-255.

———. 1957. A study of chromosome in two species of Hagfishes (Eptatretidae-Cyclostomata). *Zool. Mag.* (Japan). **66**: 29-31.

———. 1957. The chromosomes of the Japanese lancelet, *Branchiostoma belcheri*, with special reference to the sex-chromosomes. *Annot. Zool. Japon.* **30**: 42-46.

———. 1957. Chromosome Studies in Pisces, VI. The X-Y chromosomes found in *Cottus pollux* Günther (Cottidae). *Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* **13**: 289-292.

———. 1957. Chromosome Studies in Pisces, VII. A comparative study of the chromosomes in six species of the Gobiidae. *Jap. Jour. Ichthyol.* **6**: 141-146.

Oguma, K. 1930. A comparative study of the spermatocyte chromosomes in allied species of the dragonfly. *Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool.* **1**: 1-12.

———. 1934. Studies on Sauropsid Chromosomes, II. The cytological evidece proving female heterogamety in the lizard (*Lacerta vivipara*). *Arch. Biol.* **45**: 27-46.

Oguma, K. and J. J. Asana 1932. Additional date to our knowledge on the dragonfly chromosomes, with a note on occurrence of X-Y chromosome in the ant-lion (Neurpoptera). *Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool.* **1**: 133-142.

Ohmachi, F. and N. Ueshima 1957. Variation of chromosome complements in *Euscirtus hemelytrus* de Haan (Orthoptera : Gryllodea). *Bull. Fac. Agr. Mie Unive.* **15**: 1-13.

Pomini, P. 1939. Fenotipi e genotipi nei salmo italiani. *Scientia Genetica* **1**: 206-218.

Prakken, R., J. Bekendam and G. A. Pieters. 1955. The chromosomes of *Esox lucius* L. *Genetica* **XXVII**: 484-489.

Prokofieva, A. 1934. On the chromosome morphology of certain pisces. *Cytologia* **5**: 498–506.

Ralston, E. M. 1934. A study of the chromosomes of *Xiphophorus, Platypoecilus* and *Xiphophorus* × *Platypoecilus* hybrids during spermatogenesis. *Jour. Morph.* **56**: 423–432.

Retzius, G. 1890. Ueber Zellenteilung bei *Myxine glutinosa. Biol. Foren i. Stockholm, forhandl.* **II,** Nr. 8, S. 80.

Robertson, W. R. B. 1916. Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae : V-shaped chromosomes and their signficance in Acrididae, Locustidae and Gryllidae : Chromosomes and variation. *Jour. Morph.* **27**: 179–279.

Schreiber, J. 1954. Staining plant and animal chromosomes by Feulgen Aceto-Carmin sequence. *Stain Tech.* **29**: 285–292.

Schreiner, A. und K. E. Schreiner 1904. Die Reifungsteilungen bei den Wirbeltieren. Ein Beitrag zur Frage nach der Chromatinreduktion. *Anat. Anz.* **24**: 561.

——— und ———. 1904. Ueber die Entwicklung der mannlichen Geschlechtszellen von *Myxine glutinosa* (L.). I. Vermehrungsperiode, Reifungsperiode und Reifungsteilungen. *Arch. Biol.* **21**: 183.

——— und ———. 1908. Zur Spermienbildung der Myxinoiden. (Ueber die Entwicklung der männlichen Geschlechtszellen von *Mixine glutinosa* (L.). III. *Arch. Zool.* **1**: 152.

Smith, S. G. 1953. A pseudo-multiple sex-chromosome in an Indian gryllid. *Chromosoma* **5**: 555–573.

Suehiro, Y. 1951. Text-book of Ichthyology. Tokyo.

Svärdson, G. 1941. Somatic pairing in *Salmo* en *Coregonus* and its hypothetical explanation. *Ark. f. Zool. Bd. 33B.* **9**: 1–6.

———. 1945. Chromosome studies on Salmonidae. *Rep. Swedish State Inst. Freshw. Fishery Res. Drottningholm,* **23**: 1–151.

Svärdson, G. and T. Winckbom 1937. Notes on the chromosomes of some teleosts. *Hereditas* **25**: 472–476.

——— and ———. 1942. The chromosomes of two species of Anabantidae (Teleostei), with a new case of sex reversal. *Hereditas* **28**: 212–216.

Udagawa, T. 1954. Karyogram Studies in Birds, III. The chromosomes of five forms of birds. *Annot. Zool. Japon,* **27**: 91–96.

Vandel, A. 1946. Le rôle de la polyploidie dans le règne animal. *Arch. Jul. Klaus-Stift. Vererb. Forsch.* **21**: 397–410.

Vaupel, J. 1929. The spermatogenesis of *Lebistes reticulatus. Jour. Morph.* **47**: 555–587.

White, M. J .D. 1946. The evidence against polyploidy in sexually-reproducing animals. *Arm. Nat.* **80**: 610–618.

———. 1949. A cytological survey of wild population of *Trimerotropis* and *Circotettix* (Orthoptera : Acrididae). I. The chromosomes of twelve species. *Genetics* **34**: 537–563.

———. 1951. A cytological survey of wild population of *Trimerotropis* and *Circotettix* (Orthoptera : Acrididae). II. *Genetics* **36**: 31–53.

———. 1951. Structural heterozygosity in natural population of the grasshopper *Trimerotropis sparsa. Evolution* **5** :376–394.

———. 1954. Animal cytology and evolution. 2nd Ed. The University Press, Cambridge.

———. 1957a. Some general problems of chromosomal evolution and speciation in animals. *Survey of Biol. Progress, Vol.* **3**: 109–147.

———. 1957b. Cytogenetics and systematic entomology. *Ann. Rev. Entomol.* **2**: 71–90.

Wickbom, T. 1941. The sex chromosomes of Cyprinodontidae and of Teleosts in general, with a list of new chromosome numbers of Cyprinodontidae. *Ark. für Zool. Band 33 B,* **10**: 1–6.

———. 1943. Cytological studies on family Cyprinodontidae. *Hereditas* **29**: 1–24.

Wilson, E. B. 1925. The sex-chromosomes of the sea-urchin. *Science* **61**.

———. 1928. The cell in development and heredity. Macmillan Co.

Winge, Ö. 1922. A peculiar mode of inheritance and its cytological explanation. *Jour. Genet.* **12**: 137–144.

———. 1923. Crossing-over between the X and Y chromosomes in *Lebistes. Jour. Genet.*

13: 201-227.

Yamashina Y. 1943. Studies on sterility in hybrid birds. IV. Cytological researches in hybrids in the family Phasianidae. *Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool.* **8**: 307-386.

———. 1954. *Cytologia* **13**: 270-296.

Yamashina, Y. and S. Makino 1946. A study on the chromosomes of pigeon and doves. (English résumé). *Seibutu* **1**: 92-100.

Yoshida, T. H. 1957. Sex chromosmes of the tree frog, *Hyla arborea japonica*. *Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* **13**: 352-358.

Zwillenberg, L. O. and H. H. L. Zwillenberg 1954. Chromosome counts in Tunicates and a modified method for making permanent squash preparations. *Nature* **173**: 876-877.

Explanation of Plates

The figures in Plates were drawn, with the exception noted, by the aid of a camera lucida at a magnification of 3800 diameters. All the figures were reduced to approximately 2/3 for publication.

Plate I

Figs. 1—8, *Branchiostoma belcheri*. 1—3, spermatogonium. 4—6, first metaphase, (side view and polar view). 7—8, second metaphase.

Figs. 9—13, *Eptatretus burgeri*. × Ca 2300. 9—10, spermatogonium. 11—12, first metaphase. 13, second metaphase.

Figs. 14—18, *Eptatretus okinoseanus*. × Ca 2300. 14—15, spermatogonium. 16—17, first metaphase. 18, second metaphase.

Figs. 19—20, *Entosphenus reissneri*. × Ca 2300. spermatogonium.

Figs. 21—24, *Mustelus manazo*. 21—22, spermatogonium. 23—24, first metaphase.

Figs. 25—28, *Dasyatis akajei*. 25—26, spermatogonium. 27—28, first metaphase.

Figs. 29—31, *Engraulis japonicus*. 29—30, spermatogonium. 31, first metaphase.

Figs. 32, *Oncorhynchus nerka*. first metaphase.

Figs. 33, *Oncorhynchbs rhodurus*. first metaphase.

Figs. 34—35, *Oncorhynchus masou*. 34, first metaphase (andromonus form). 35, first metaphase (land locked form).

Figs. 36, *Oncorhynchus keta*. first metaphase.

Figs. 37—38, *Salmo irideus*. 37, spermatogonium. 38, first metaphase.

Figs. 39, *Salvelinus fontinalis*. first metaphase.

Figs. 40—41, *Salangichthys microdon*. first metaphase.

Plate II

Figs. 42—45, *Rhodeus ocellatus*. ×3000. 42—43, spermatogonium. 44, first, metaphase. 45, second metaphase.

Figs. 46—60, *Acheilognathus rhombea*. 46—49, 4V-type complex. 46—47, spermatogonium. 48, first metaphase. 49, senond metaphase. 50—53, 2V-type complex. 50—51, spermatagonium. 52, first metaphase. 53, second metaphase. 54—57, NO V-type complex. 54—55, spermatogonium. 56, first metaphase. 57, second metaphase. 58—60, serially aligned arrangements of the paired chromosomes. × Ca 5200. 58, 4V-type complex (from Fig. 46); 59, 2V-type complez (from Fig. 51); 60, NO V-type complex (from Fig. 55).

Figs. 61—63, *Acheilognathus limbata*. ×3000. 61—62, spermatogonium. 63, first metaphase.

Figs. 64—66, *Acheilognathus cyanostigma*. 64—65, spermatogonium. 66, first metaphase.

Figs. 67—68, *Pungtungia herzi*. × Ca 3300. spermatogonium.

Figs. 69—72. *Sarcocheilichthys variegatus.* 69, spermatogonium. 70—71, first metaphase. 72, second metaprase.
Figs. 73—76. *Abbottina rivularis.* 73—74, spermatogonium. 75, first metaphase. 76, second metaphase.
Figs. 77 -79. *Gnathopogon elongatus elongatus.*
77—78, spermatogonium. 79, first metaphase.
Figs. 80—82. *Gnathopogon elongatus caerulescens.*
80—81, spermatogonium. 82, first metaphase.

Plate III

Figs. 83—85. *Pseudogobio esocinus.* ×3000. 83, spermatogonium. 84, first metaphase. 85, second metaphase.
Figs. 86—88, *Hemibarbus longirostris.* 86—87, spermatogonium. 88, first metaphase.
Figs. 89—91. *Ctenopharyngodon idellus.* 89—90, spermatogonium. 91, first metaphase.
Figs. 92—94. *Moroco steindachneri.* 92—93, spermatogonium. 94, first metaphase.
Figs. 95—96. *Moroco percnurus.* 95, first metaphase. 96, second metaphase.
Figs. 97—100. *Zacco platypus.* ×3000. 97—98, spermatogonium. 99, first metaphase. 100, second metaphase.
Figs. 101—102. *Zacco temmincki.* × Ca 3300. spermatogonium.
Figs. 103—106, *Opsariichthys uncirostris.* 103—104, spermatogonium. 105, first metaphase. 106, second metaphase.
Figs. 107—109, *Hemigrammocypris rasborella.*
107—108, spermatogonium. 109, first metaphase.
Figs. 110—112, *Ishikauia steenackeri.* 110—111, spermatogonium. 112, first metaphase.
Figs. 113—115. *Barbus semifaciolatus.* ×3000.
113—114, spermatogonium. 115, first metaphase.
Figs. 116—119, *Lefua echigonia.*
116—117, spermatogonium. 118, first metaphase. 119, second metaphase.
Figs. 120—122, *Cobitis biwae.* 120—121, spermatogonium. 122, first metaphase.

Plate IV

Figs. 123—129. *Parasilurus asotus.* ×3000. 123—124, spermatogonium. 126, first metaphase. 125, side view of first metaphase. 127, second metaphase.
Figs. 128—130, *Pelteobagrus nudiceps.* 128—129, spermatogonium. 130, first metaphase.
Figs. 131—133, *Echelus uropterus.* 131—132, spermatogonium. 122, first metaphase.
Figs. 134—136, *Muraena pardalis.* 134—135, spermatogonium. 136, first metaphase.
Figs. 137—140, *Gymnothorax kidako.* 137—138, spermatogonium. 139—140, first metaphase.
Figs. 141—143. *Coreoperca kawamebari.* 141—142, spermatogonium. 143, first metaphase.
Figs. 144—150. *Sillago sihama.* 144—145, spermatogonium. 146, side view of first metaphase. 147—148, polar view of first metaphase. 149, side view of first anaphase. 150, second metaphase.
Figs. 151—154, *Cilias pulchella.* 151—152, spermatogonium 153—154, first metaphase.
Figs. 155—159, *Callionymus richardsoni.* 155—156, spermatogonium. 157, serially aligned arrangement of the paired chromosomes (from of Fig. 155). 158—159, first metaphase.

Plate V

Figs. 160—164, *Mogrunda obscura.* 160—161, spermatogonium. 162, nucleus at early leptotene stage, indicating the chromatin nucleolus (XY-element). c, chromatoid body. P, plasmosome. 163—164, nuclei at late leptotene stage. 165—166, the same at pachytene. 167, nucleus at diakinesis. 168—170, first metaphase.

168—169, polar views, showing 30 bivalents and a sex-bivalent (XY-complex). 170, side views of the first division, showing XY-complex. 171—172, XY-complex in side view. 173—174, second metaphase.

Figs. 175—179, *Tridentiger obscurus*. ×3000. 175—176, spermatogonium. 177, first metaphase. 178—179, first metaphase, including univalent chromosomes (21 bivalents+2 univalents).

Figs. 180—182. *Gobius similis*. 180—181, spermatogonium. 182, first metaphase.

Figs. 183—185, *Gobius abei*. 183—184, spermatogonium. 185, first metaphase.

Figs. 186—189. *Acanthogobius flavimanus*. ×3000. 186—187, spermatogonium. 188, first metaphate. 189, second metaphase.

Figs. 190—192, *Chaenogobius urotaenia*. 190—191, spermatogonium. 192, first metaphase.

Figs. 193—195, *Chaenogobius isaza*. 193—194, spermatogonium. 195, first metaphase.

Figs. 196—198, *Boleophthalmus pectinirostris*. 196—197, spermatogonium. 198, first metaphase.

Figs. 199—201, *Periopthalmus cantonensis*. 199—200, spermatogonium. 201, first metaphase.

Figs. 202—204, *Pseudolabrus japonicus*. 202—203, spermatogonium. 204, first metaphase.

Plate VI

Figs. 205—208, *Stephanolepis cirrhifer*. 205—206, spermatogonium. 207, first metaphase. 208, second metaphase.

Figs. 209—211, *Rudarius ercodes*. 209—210, spermatogonium. 211, first metaphase.

Figs. 212—214, *Novodon modestus*. 212—213, spermatogonium. 214, first metaphase.

Figs. 215—230, *Inimicus japonicus*. 215—216, spermatogonium. 217, first metaphase. 218—220, first metaphase plate, including univalent chromosomes. 218, 24 bivalents +2 univalents. 219, 23 bivalents+4 univalents. 220, 22 bivalent+6 univalents. 221—224, side view of first metaphase, including univalent chromosomes. 225, formation of a small nucleus of univalents. 226—230, second metaphase. 226, 23 dyads. 227, 24 dyads. 228, 25 dyads. 229, 26, dyads. 230, 27 dyads.

Figs. 231—233, *Hexagrammos otakii*. 231—232, spermatogonium. 233, first metaphase.

Figs. 234—243, *Cottus pollux*. 234—235, spermatogonium. 236, nucleus at early leptotene stage, indicating the heteropycnotic nucleolus (XY element). P, plasmosome. 237—238, nuclei at late leptotene stage. 239—240, the same at pachytene. 241, polar view of the first metaphase. 242, sides view of the first metaphase. 243, second metaphase.

Figs. 244—245, *Paralichthys olivaceus*. 244, spermatogonium. 245, first metaphase.

Figs. 246—248, *Limanda yokohamae*. 246—247, spermatogonium. 248, first metaphase.

Figs. 249—251, *Kareius bicoloratus*. 249—250, spermatogonium. 251, first metaphase.

Plate I

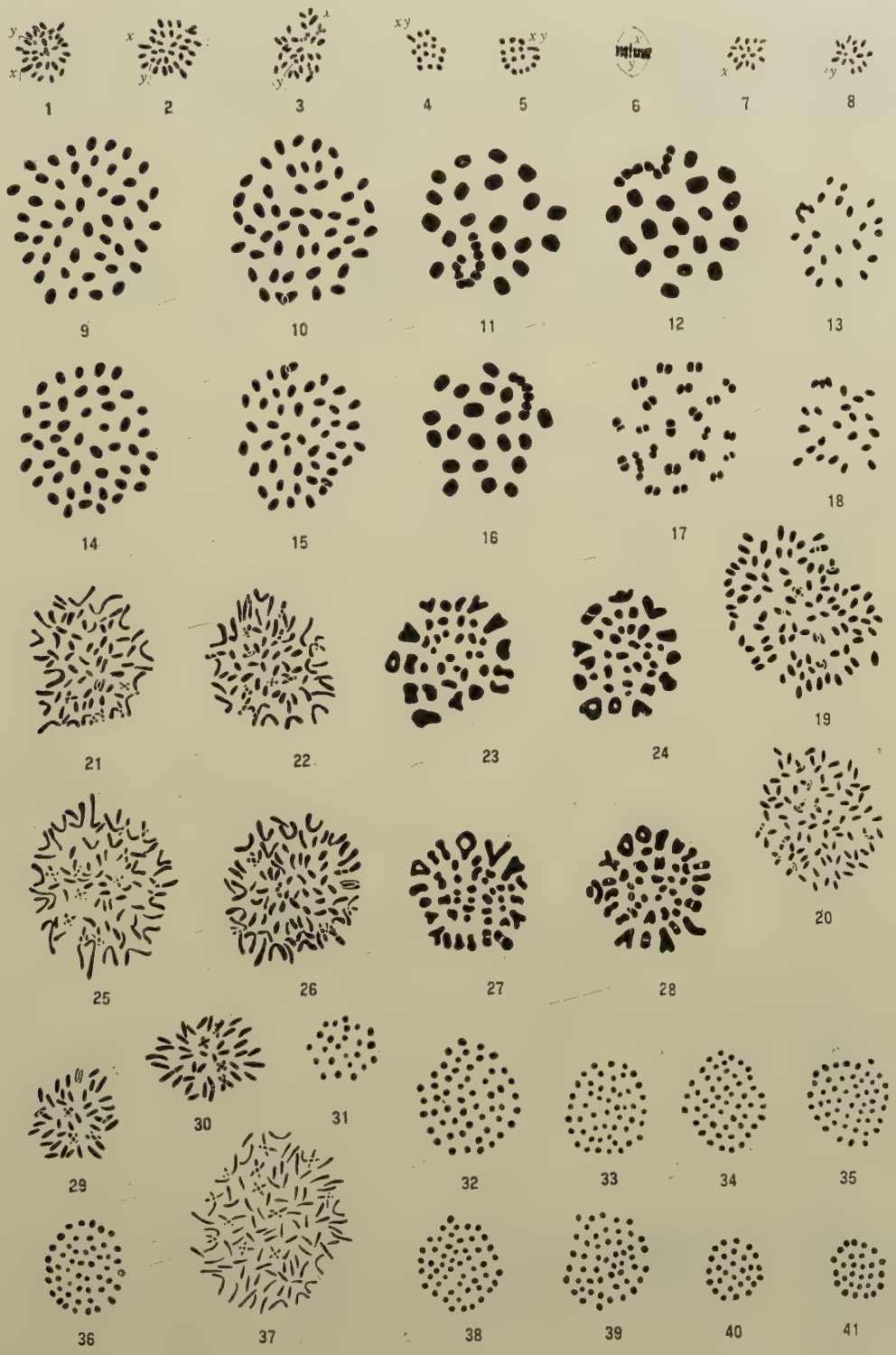

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

Plate II

42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82

Plate III

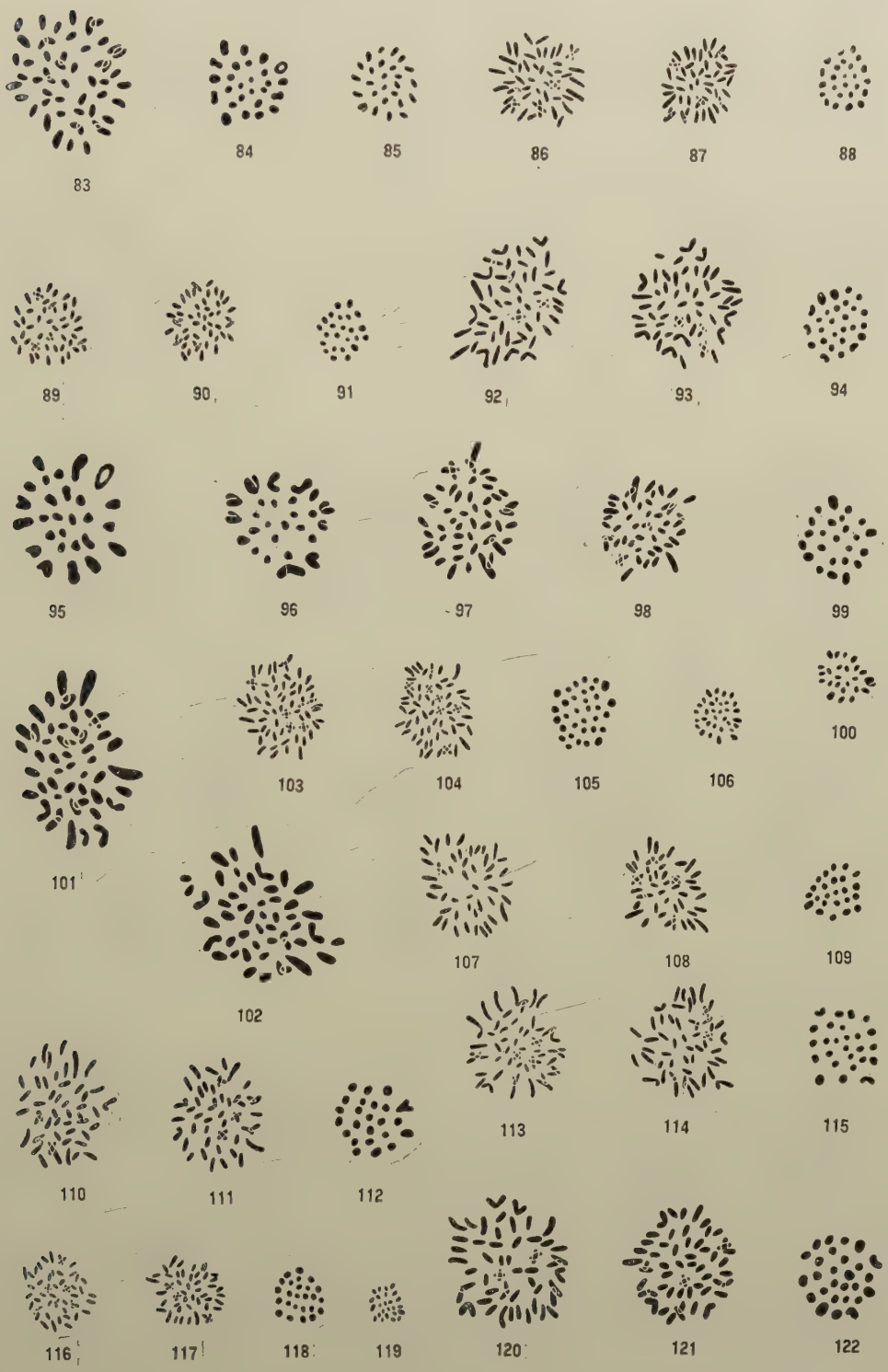

83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122

Plate IV

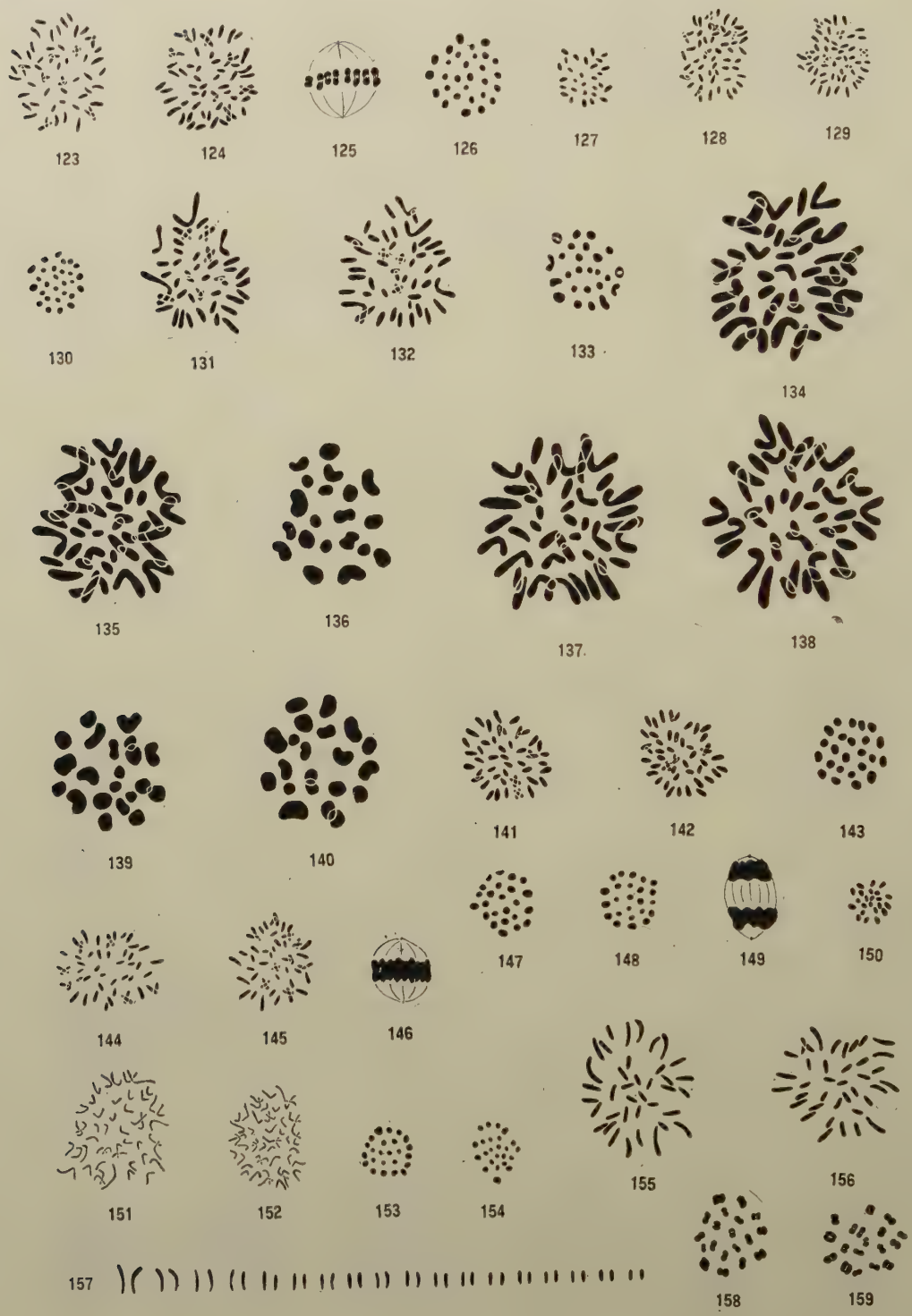

123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159

Plate V

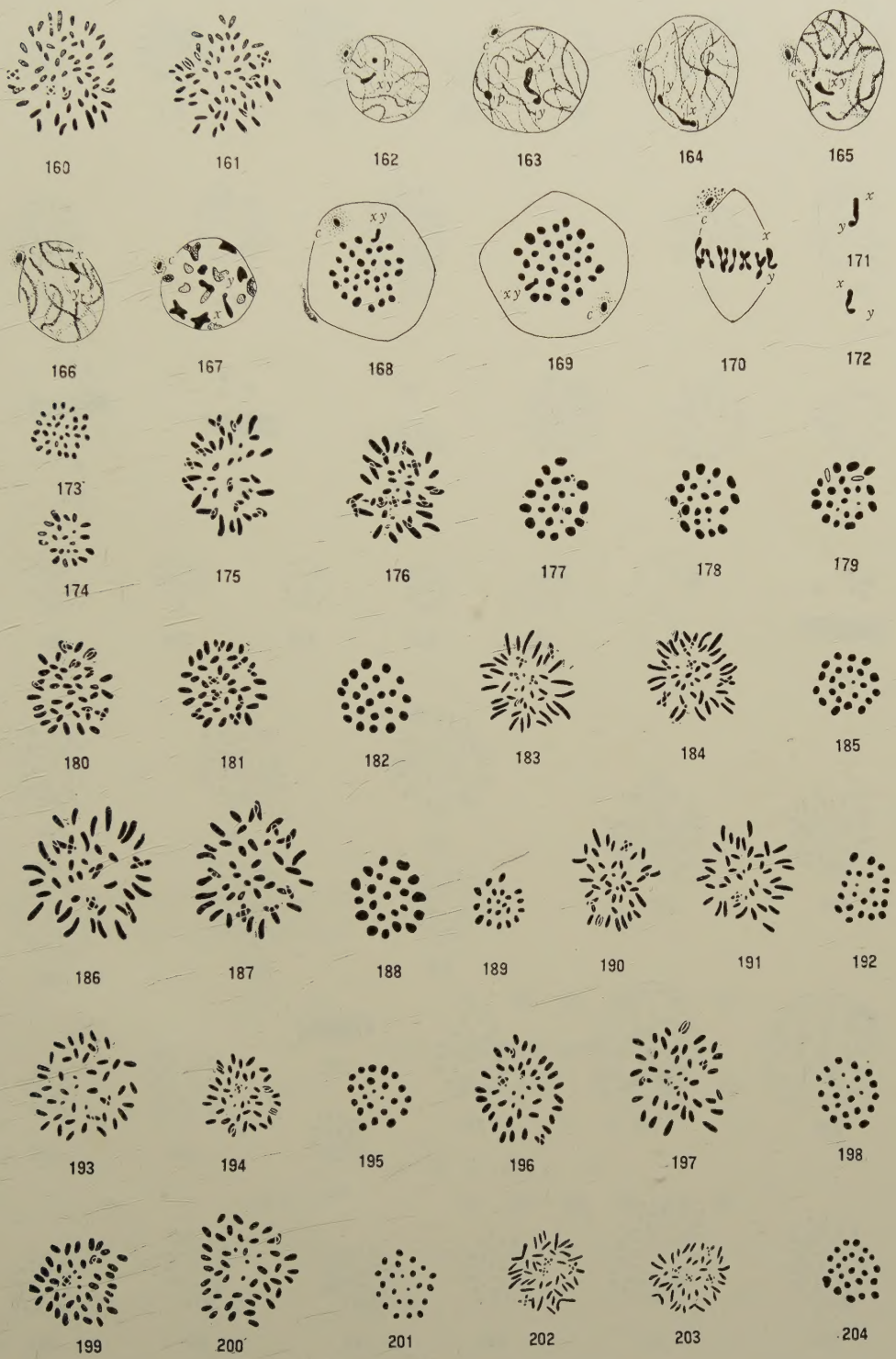
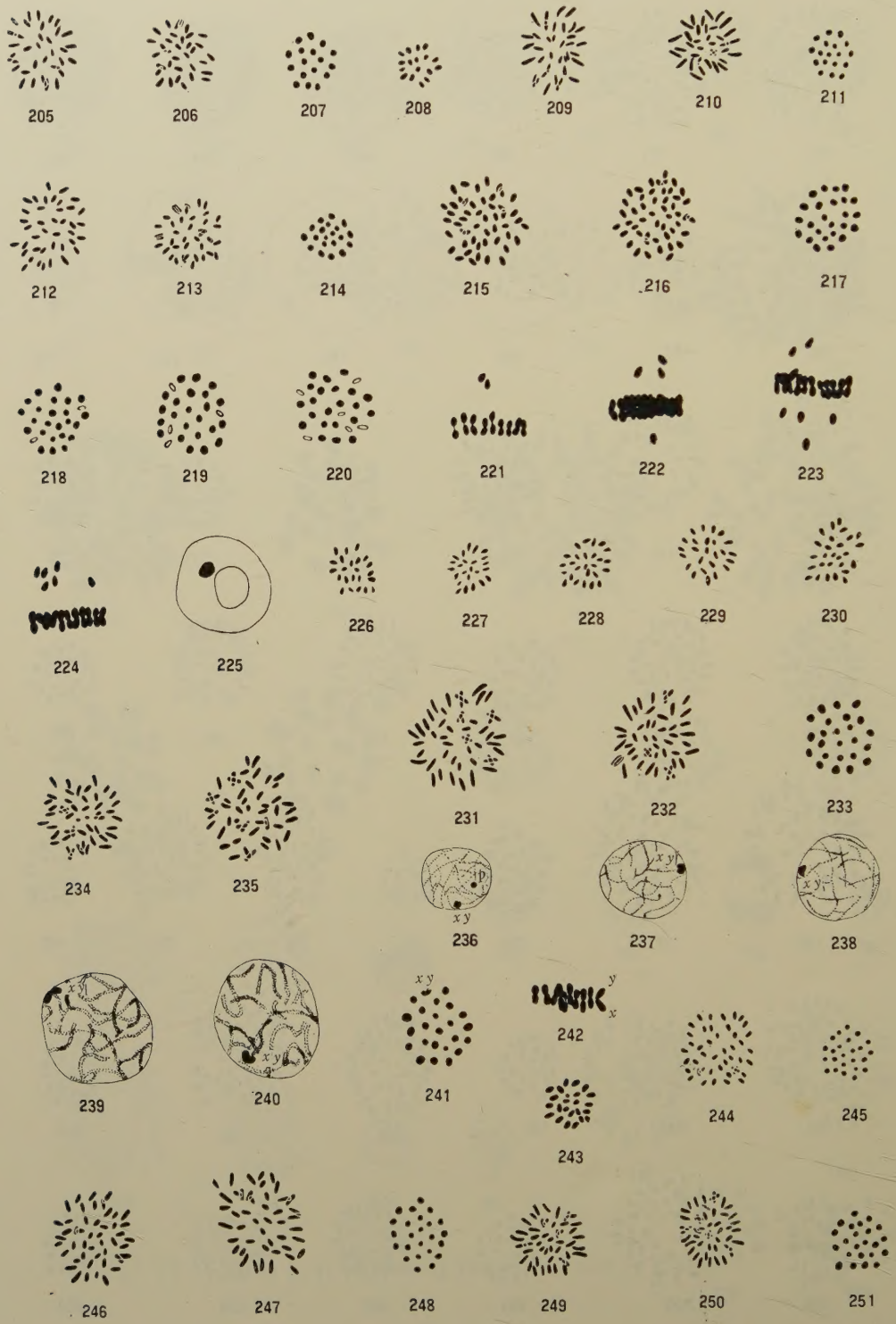

160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204

Plate VI


205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251

昭和35年3月10日印刷
昭和35年3月25日発行

編　者　兵庫農科大学

大阪市都島区野田町43
印刷所　株式会社 天 業 社
出 間 照 久

兵庫県篠山町
発行所　兵庫農科大学